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REMOVAL OF VIRUS FROM PUBLIC WATER SUPPLIES

by

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16. ABSTRACT

Assays of Missouri River water at Lexington, MO., for the presence of naturally-occurring human enteroviruses resulted in the recovery of poliovirus, Coxsackie and ECHO viruses. Virus concentrations were found to be highest in the winter and spring, and there ~~resulting~~ inverse correlat~~ions~~al with conventional microbiological indicators of water contamination. No virus isolates were obtained from 65 samples of finished water from the Lexington water treatment plant.

Batch disinfection studies of raw Missouri River water indicated that chlorine dioxide and ozone were most effective in inactivating viruses whereas chlorine and chloramine were only marginally effective at dosages commonly employed in water treatment.

Studies of virus removal by physical removal processes indicated that a one- to two-order of magnitude reduction in viruses was attained by physical removal processes alone. A comparison of conventional treatment versus direct filtration indicated that comparable removals could be obtained by both processes, although conventional treatment was more consistent in performance. Reductions in other microbial indicators paralleled virus removals indicating the effectiveness of the indicators in monitoring virus removals.

Comparisons of chlorine and chloramine in pilot plant studies using Missouri River water showed both disinfectants were capable of producing a finished water than met bacteriological standards. Chloramine, however, proved to be more stable than chlorine in achieving lower standard plate counts.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, four water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

Viruses discharged from wastewater treatment plants constitute one of the most difficult challenges to the integrity of public water supplies. This study attempts to evaluate the effectiveness of current water treatment and disinfection techniques in meeting that challenge.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

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ABSTRACT

Concentration of raw Missouri River water and assays for the presence of naturally occurring human enteroviruses resulted in the recovery of polio, Coxsackie, and ECHO viruses. Virus concentrations were found to be highest in the winter and spring, when river flows (and therefore the effects of dilution) were lowest. Statistically significant inverse correlations were found between virus recoveries and conventional microbiological indicators of water contamination (total coliform, fecal coliform, standard plate counts).

An evaluation of the effectiveness of the water treatment plant at Lexington, Missouri, indicated that presedimentation plus the addition of lime resulted in the reduction of virus levels. Concentrates derived from 1.89 m^3 (500 gallon) samples of treated drinking water were assayed in the U.S. EPA Health Effects Research Laboratory. No isolates were obtained from the 65 samples analyzed.

Batch disinfection studies were conducted using raw Missouri River water treated with chlorine, chloramine, chlorine dioxide, or ozone. Though data were limited, only the chlorine dioxide and ozone appeared capable of inactivating viruses and other indicators of microbial contamination. Chlorine and chloramine were only marginally effective at dosages commonly employed in water treatment.

Studies of the effectiveness of physical removal processes were conducted using water treatment pilot plants constructed of stainless steel and housed in a 12.8 m (42 ft.) tractor trailer. Owing to insufficient numbers of raw water viruses, bacteriophage-seeded studies were conducted that indicated that a one to two-order of magnitude reduction in viruses was obtained by physical removal processes alone. A comparison of conventional treatment versus direct filtration indicated that comparable removals could be obtained by both processes, although conventional treatment was somewhat more consistent in performance. Reductions in other microbial indicators paralleled virus removals indicating the effectiveness of the indicators in monitoring virus removals.

The final portions of the study was conducted by the Capital City Water Treatment Plant in Jefferson City, Missouri. Chlorine and chloramine were applied in parallel pilot plant units for the disinfection of Missouri River water. Both disinfectants were capable of producing a finished water that met bacteriological standards. Chloramine, however, proved to be more stable than chlorine in achieving lower standard plate counts.

This report was submitted in partial fulfillment of Grant No. R-804903-02, by the University of Missouri under the sponsorship of the U.S. Environmental Protection Agency. The report covers the period October 18, 1976, to October 17, 1979, and work completed as of July 17, 1980.

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SECTION I

INTRODUCTION

One of the most disturbing observations resulting from virological investigations is the fact that many viruses, including the polio and Coxsackie enteroviruses, may be found in both ground and surface water sources. Domestic sewage discharges are currently accepted as the primary mode for viral contamination of the aquatic environment. Although surface water sources are estimated to contain a maximum of 18 percent sewage, more wastewater is being recycled each day, thereby increasing the threat of water borne virus disease.

Little is known about the prevalence, distribution, and removability of naturally-occurring viruses in the aquatic environment. From the standpoint of public health protection, it is generally believed that conventional water treatment practices (coagulation/flocculation, sedimentation, filtration, and disinfection) reduce infectious virus concentrations to levels far below the sensitivity of current analytical methods. It has, however, been indicated by several investigators (Berg et al., Engelbrecht et al., and Liu et al.) that viruses entering a water treatment plant have greater resistance to chemical disinfectants compared with most microorganisms, including those used as indicators of pollution.

Worst case estimates lead to the prediction that one infectious virus unit might be present in each 400,000 liters of drinking water (26). In a population of 100,000 persons consuming 1 liter of "uncooked" water per person per day, one infectious virus unit would be ingested every 4 days. The quantity of clinical disease resulting from such exposures is unknown, but it is important to observe that children under age 10 (20% of the U.S. population) appear to be the most susceptible to viral infection and that an estimated 38 percent of all children ages 1 to 4 do not receive primary vaccination. Inasmuch as viral infection has been shown to result from low numbers of ingested units, viral disease via the water route may pose a subtle yet significant challenge to public health.

The Present Study

Amendments to the Safe Drinking Water Act made the U.S. Environmental Protection Agency (EPA) directly responsible for determining evaluating modes of aquatic virus contamination and for assessing the potential threat to public health. This congressional directive implied the necessity for a comprehensive tracking of viruses from the infected individual, and for evaluating the potential threat to public health.

In response to their appointed task, EPA instituted a number of programs to explore the many questions related to viruses in the environment. One of the immediate objectives for inhouse research was to conduct a nationwide survey to evaluate the occurrence of viruses in community drinking water systems. After 3 years, the analysis of 119 finished water samples from 42 locations yielded no virus recoveries. Although these results indicated that public health was in no imminent danger from virus contamination, more definitive information was required before health safety could be assured. It was determined that longitudinal data concerning raw source water virus levels were needed to augment data from treated water analyses. In addition, research efforts were required to observe the virus removal efficiency of individual water treatment unit processes.

Supplementary raw water microbiological samples collected in conjunction with the finished water virus concentrates showed that the Missouri River downstream from Kansas City, Missouri contained the heaviest domestic waste pollution of all source water locations sampled. Fecal coliform densities ranging from 2,300 to 16,000 organisms per 100 ml indicated that the Missouri River below Kansas City was likely to contain substantial levels of naturally occurring viruses, therefore posing the greatest challenge to downstream water treatment plants. Preliminary investigations determined that the Lexington, Missouri, water treatment plant would provide the most strategic location for a longitudinal study of naturally occurring human enteroviruses.

Project Objectives

The objectives of the research proposed by the University of Missouri-Columbia, Department of Civil Engineering were to quantitatively determine naturally occurring human enterovirus levels in a raw drinking water source and to determine the capability of various water treatment unit processes to remove and/or destroy these viruses. The investigation would be conducted at the nearly century-old Lexington, Missouri, water treatment plant, utilizing water from the Missouri River. Ancillary questions relating to the use of conventional microbiological indicators as surrogates for the presence of virus contamination or as an index of the removal of virus by water treatment processes were to be investigated.

Using batches of Missouri River water, the study was also to determine the effectiveness of the water disinfectants; chlorine, chloramine, chlorine dioxide and ozone, in inactivating naturally occurring enterovirus.

Finally, a mobile pilot water treatment facility was to be constructed to simulate conventional physical treatment processes. This facility would permit an evaluation of the effectiveness of individual physical removal processes, independently, for the removal of virus. By these means, some preliminary assessment of the relative protection afforded by chemical disinfection or by the physical removal of particles through conventional water treatment might be made. As part of the study, modifications were made to the pilot plant facility to permit a comparison of conventional water treatment with direct filtration. Considering the high suspended solid content of the raw water source, this was a strong challenge to the direct filtration process.

Upon completion of the studies at Lexington, Missouri, the mobile water treatment facility was taken to Jefferson City, Missouri, and placed in operation on the site of the Capital City Water Treatment Plant. Treating raw Missouri River water with identical parallel treatment schemes, the pilot plant facility made it possible to compare the results of disinfecting with chlorine (hypochlorous acid) and chloramine on the microbiological quality of the finished drinking water. In addition, the formation of chlorinated organic byproducts was observed by monitoring total trihalomethanes (TTHM). Removals of total organic carbon (TOC) and organic compounds which had trihalomethane-forming potential (THMFP) were also observed. This comparative study was of special interest to water suppliers who derive water from the Missouri River since virtually all such supplies have been disinfected using chloramine, intentionally or inadvertently, for decades.

In summary, the present study consisted of the following parts and objectives:

I. Longitudinal Study of Virus in Missouri River

(1) to enumerate and identify human intestinal viruses in a drinking water source receiving upstream discharges of partially-treated wastewater.

(2) to observe the variation in virus levels with season, water temperature, river flow and other conditions over, at least, one calendar year.

(3) to determine the utility of conventional and proposed microbiological parameters as indicators of virus populations.

II. Virus Removal at Lexington, Missouri

(1) to observe the effectiveness of the water treatment processes employed at the Lexington water treatment plant in removing or inactivating viruses in Missouri River water.

(2) to monitor the finished drinking water at Lexington to determine whether whether virus particles were able to penetrate through the plant into the distribution system.

III. Chemical Disinfection for Virus Inactivation

(1) to observe the effectiveness of chlorine, chlorine plus ammonia, chlorine dioxide, and ozone for the inactivation of virus.

(2) to compare the reduction of microbiological parameters with virus inactivation as a function of disinfectant dosage and time.

IV. Physical Removal of Virus Particles

(1) to design and fabricate a mobile water treatment pilot plant facility to simulate conventional water treatment processes.

(2) to utilize the pilot plant to observe the removal of virus particles from Missouri River water at Lexington, Missouri, in comparison with the reductions in indicator microorganisms.

V. Chlorine Versus Chloramine for Water Disinfection

(1) to observe the effectiveness of chlorine and chloramine for the disinfection of Missouri River water in conjunction with conventional water treatment.

(2) to study the effect of chlorine and chloramine on the formation of trihalomethanes.

SECTION 2

CONCLUSIONS

I. Longitudinal Study of Virus in Missouri River Water

Naturally-occurring human enteroviruses were found in Missouri River water at the Lexington treatment plant intake throughout the study. An average of 2.5 viruses were recovered in 216 samples of approximately 100 gallons (0.38 cubic meters) each. Polioviruses were most abundant (69%), followed by Coxsackie (11%) and ECHO viruses (5%). Of the total, only 10% of the virus particles were recovered on the 3-5 micrometer pore size prefilters, indicating that most of the recovered virus particles were not associated with the suspended sediment in Missouri River water.

Virus concentrations were found to average 10 plaque-forming units (PFU) per 100 gallons in the winter and spring of 1977, 1.5 PFU/100 gallons in the summer and fall and 5 PFU/100 gallons in the winter and spring of 1978. When the virus concentrations were normalized with respect to river flow, the resulting "virus flow" in the Missouri River at Lexington appeared less variable throughout the year. However, seasonal winter and spring "virus flow" peaks were still apparent.

Statistically significant inverse correlations were obtained between concentrations of virus and microbiological indicators (fecal coliform, total coliform, standard plate counts). These results, coupled with winter and spring "virus flow" peaks, indicate that conventional microbial parameters will not prove useful as indicators of virus contamination in the Missouri River.

II. Virus Removal at Lexington, Missouri

Samples were taken of the presettled, lime-treated and finished water at the Lexington, Missouri, water treatment plant over the period of December 2, 1976 to March 22, 1978. In 21 samples taken of presettled water, a total of 39 virus were recovered. Seven virus were recovered from 14 samples taken after the addition of lime to the raw water. Finally, no virus were recovered from the 71 finished water samples collected.

The results of the survey of plant performance would indicate that the overall water treatment process at Lexington, Missouri, is consistently effective in reducing virus to below detectable limits. Moreover, preliminary treatment of raw Missouri River water with lime appeared to result in a measurable inactivation of the virus present.

III. Chemical Disinfection for Virus Inactivation

The disinfectants (chlorine, chloramine, chlorine dioxide, and ozone) each showed the potential for inactivating the human enterovirus present in raw Missouri River water when sufficient dosage and time was provided. In general, effective virucidal action was accompanied by near-total destruction of fecal and total coliform as well as by two-to-three order of magnitude reductions in standard plate counts. The comparatively low numbers of acid-fast bacteria and yeast found in Missouri River water appears to limit their utility as indicator organisms.

IV. Physical Removal of Virus Particles

As part of the study of the physical removal of virus-sized particles from Missouri River water, a mobile water treatment research facility was constructed. Twin water treatment pilot plants were constructed, and mounted in a tractor-trailer. A separate, limited-edition internal report, entitled "Construction Plans and Operations Manual for USEPA Mobile Water Treatment Facility" has been prepared to provide subsequent operators of the pilot plant facility with design calculations, shop drawings, fabrication notes, plus operation, maintenance and safety instructions.

Bacteriophage MS_2, were seeded to raw Missouri River water as surrogates for human enteroviruses in the physical removal studies in order to increase the number of virus particles present, to shorten the time between sampling and receiving the results of culture analysis and to reduce costs.

Coagulation with aluminum sulfate, followed by sedimentation and dual-media filtration resulted in an overall removal of 93% of seeded bacteriophage. Comparable removals were obtained with respect to fecal and total coliform, fecal streptococcus and standard plate count organisms.

Coagulation with a cationic polyelectrolyte followed by direct filtration resulted in an 87% removal of seeded bacteriophage. Using direct filtration, results were more erratic (57-99%) than those obtained with conventional treatment (85-99%). Again, the removal of other indicator organisms paralleled the removal of MS_2 bacteriophage.

Turbidity reductions reflected organism removals. Influent turbidities ranged from 19 to 180 NTU. Effluent turbidities ranged from 1-7 NTU for conventional treatment. Direct filtration produced turbidities in the range of 0.4-8.5 NTU, except for a single high value of 77 NTU. This turbidity breakthrough occurred during a period of high influent turbidity.

Overall, it appeared that a one to two order of magnitude removal of seeded virus and other microbial indicators of pollution could be obtained by both conventional water treatment and direct filtration under proper operating conditions. Direct filtration, however, is more subject to upset during periods of high influent turbidities.

V. Chlorine Versus Chloramine for Water Disinfection

Studies of organism removal and disinfection by chlorine and chloramine at Jefferson City, Missouri, began with a comparison of organism reductions with and without the addition of chlorine.

Aluminum sulfate coagulation and sedimentation removed 80-85% of the standard plate count organisms. With filtration, organism removal increased to 90%. The addition of chlorine increased organism reductions to 99.9% and greater.

The subsequent comparison of chlorine versus chloramine, each added prior to the rapid mix tank, indicated that each of the two disinfectants were effective in attaining bacteriological drinking water standards. Moreover, chloramine produced an order of magnitude lower trihalomethanes.

With the inclusion of granular activated carbon post-adsorbers in the pilot plant operation at Jefferson City, Missouri, it was possible to observe the removal of trihalomethanes as well as the reduction in total organic carbon and substances which have trihalomethane-forming potential (THMFP). Finally, organism growth in granular activated carbon post-adsorbers was observed by monitoring effluent microorganisms.

Overall, it appeared that comparable disinfection could be achieved, with chlorine and chloramine, while effluent trihalomethanes were considerably lower using chloramine than could be achieved by using the combination of chlorine and granular activated carbon.

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SECTION 3

RECOMMENDATIONS

Continuing efforts should be directed at simplifying and improving the sensitivity of virus concentration and assay techniques. The principal limitation on monitoring human enterovirus in natural waters and observing their removal by various water treatment processes is inadequate sensitivity of the virus analysis. The efficiency of virus recovery from natural water sources may presently be quite low, depending on virus type, concentration, and sample size. The number of virus actually present in a natural water sample may therefore be considerably greater than the number recovered.

At the very least, long-term monitoring of human enteroviruses in water supply source waters should be conducted at several sites in the United States to determine the variation in virus levels due to virus disease outbreaks, floods, droughts, wastewater treatment breakdowns, increased wastewater reuse, or other factors that may influence virus contents of natural waters. At present, no water supply in the United States has accumulated as much as a decade of virus analysis data. The vast majority have never performed a single virus analysis on their raw water source.

SECTION 4

MATERIALS AND METHODS

LOCATION OF TREATMENT FACILITIES

The Lexington, Missouri water treatment plant is located approximately 80 river kilometers downstream of Kansas City, Missouri. With an average river flow of 990 m^3/s, travel time from the metropolitan Kansas City area to Lexington requires 15 hours.

The river stretch between Kansas City and Lexington, Missouri receives domestic waste discharges totaling 6.5 m^3/s. The single largest discharge originates from the Blue River treatment facility located in southeast Kansas City. Influent to the Blue River wastewater treatment plant averages 2.8 m^3/s and obtains only primary treatment, without chlorination, prior to discharge. All other domestic waste treatment facilities upstream of the intake to the Lexington water treatment plant (WTP) provide secondary treatment or pond stabilization before discharge.

In addition to domestic wastes, the 80 kilometer Missouri River stretch receives a variety of industrial discharges. The largest of these volumes result from power plant cooling waters. However, other wastes are discharged from the pulp and paper, petrochemical, metal products, sand and gravel, cement, and food processing industries.

WATER TREATMENT PROCESSES

The original Lexington plant was constructed in 1884 and consisted solely of sedimentation and pumping facilities. Chlorination was added some time later. In 1936 the plant was expanded to incorporate lime softening and sand filtration. Just prior to the expansion, the facility was offered for sale to the community of Lexington for $50,000 but was rejected. The present value of the system is in excess of $1.2 million.

The physical layout and treatment facilities have remained much the same since 1936. Manually cleaned, earth-bottom basins are still utilized for the majority of the water treatment.

The Missouri River is the sole water source for the Lexington plant. This factor was one of the primary reasons for the selection of the plant for the virus investigation. Raw river water is supplied to the plant through a 200 mm pipe extending to an intake structure positioned some 90 meters off the river bank. The intake system is protected by a dike constructed of placed stones. An emergency surface withdrawal connection is maintained in the event of damage to the intake structure due to ice or flood waters.

Water treatment "normal" for the Missouri River Basin begins as the raw river water enters Basin #1 for presedimentation. With the average system flow of 1300 m^3/day, the theoretical residence time for presedimentation is estimated to be 22.5 hours. Lime is added to the effluent of Basin #1 increasing the pH to 10 and resulting in the precipitation of calcium carbonate. After a 19 hours detention period, chlorine and ferrous sulfate are added as coagulants. In routine operations Basin #3 is used as an auxiliary unit and, therefore, bypassed. The coagulated effluent from Basin #2 is piped directly to Basin #4 where turbidity and additional calcium carbonate reduction is achieved through flocculation and 40 hours of sedimentation.

Final treatment is provided by two 3.6 m x 4.5 m filters containing 0.6 m of sand media overlaying 0.8 m of gravel. Post-filter chlorination is applied through a 22 kg capacity chlorinator at a rate of 5 kg/day. An ammonium source is added to the chlorinated effluent (via ammonium sulfate solution) to produce a combined chlorine residual ranging from 2 to 2.5 mg/l.

VIRUS ASSAY

Virus sampling and analysis was subcontracted to the Carborundum Corporation, Niagara Falls, New York. The actual virus recovery procedure was implemented with the Carborundum "Aquella" virus concentrator. This proprietory apparatus is designed on the same principles and procedures as outlined in Section 913, Standard Methods for the Examination of Water and Wastewater, 14th edition (1). In brief, the system consists of low pH, aluminum hydroxide sorption combined with a patented multi-cartridge membrane filtration unit. In comparative testing using raw Missouri River water, the "Aquella" system exhibited consistency and sensitivity for enterovirus recovery equivalent to methods utilized by the EPA.

Following sampling, virus concentrates were packed in dry ice and shipped via air express to the Carborundum-affiliated laboratories at the University of New Hampshire. Virus assay proceeded with inoculation of the sample concentrates into Buffalo Green Monkey Kidney and Primary Monkey Kidney tissue cultures and incubation for 2 to 3 weeks at 37°C. Resulting plaques were "picked" and recultured to confirm viral etiology. Second generation plaques were identified by serum neutralization. The virus assay procedure was sensitive for the following enteroviruses: Polio type I, II and III; Coxsackie B types 1 through V and ECHO viruses of all types. Detailed information on both the virus concentration and assay procedures is presented in Appendix A.

PHYSICAL AND CHEMICAL ANALYSES

Physical and chemical analyses of raw and treated Missouri River water consisted of: pH, temperature, turbidity, alkalinity and hardness. All parameters were measured on site in conjunction with each virus sampling using field analysis techniques in accordance with Standard Methods (1). Raw water turbidities below 40 NTU were measured utilizing a Hach turbidimeter whereas turbidities exceeding 40 NTU were measured by Lexington treatment plant personnel using a Jackson candle apparatus. This was necessitated by plant operational changes during periods of high turbidity.

MICROBIOLOGICAL ANALYSES

Microbiological analyses included total coliform, fecal coliform, standard plate count, yeast and acid-fast organism enumerations. Samples were collected at the start of each virus concentration procedure and shipped by bus to the University of Missouri-Columbia Civil Engineering Laboratories. All sample collections and analyses followed Standard Method Procedures (1), except for those for yeast and acid-fast bacteria which were performed utilizing the procedures described by Engelbrecht, et. al. (2). The transit times for samples, i.e., time elapsed between sample collection and analysis at UMC laboratories ranged from 6 to 18 hours.

SECTION 5

RESULTS AND DISCUSSION

LONGITUDINAL STUDY OF VIRUS IN MISSOURI RIVER

Various standard physical, chemical and microbiological parameters of raw Missouri River water were measured in conjunction with each virus analysis. It was hoped that one or several of these parameters would correlate with and serve as an indicator of virus populations in the Missouri River. Graphical presentations of the collected raw river water data are given in Figures 1 through 8 for the period December, 1976 through December, 1978. This particular time period corresponds to consecutive virus sampling dates over the entire two-year longitudinal study.

Physical Parameters

Figures 1, 2 and 3 exhibit, respectively, the physical river water characteristics of flow, temperature and turbidity. The Missouri River flow data presented in Figure 1 was obtained from the U.S.G.S. Water Resources Division in Rolla, Missouri. The flow data was recorded at the Kansas City, Missouri gauging station located approximately 80 kilometers upstream from the Lexington, Missouri site. Hydrologists for the U.S.G.S. indicated that the large volume of the Missouri River makes flow differences between the Lexington site and the Kansas City station insignificant because of limited accuracy of the gauging equipment.

Missouri River Flow

Figure 1 shows that the flow in the Missouri River is rather well regulated, aside from periods of high storm water run-off. Low winter flows average 20,000 cfs and gradually increase during the spring months to attain an average high summer flow of 70,000 cfs. Flow regulation is supervised by the U.S. Army Corps of Engineers in order to provide sufficient water levels for river navigation beginning in the middle of spring and extending through the late fall. Such a controlled river flow condition has significant effects on many of the physical, chemical, microbiological and viral raw water characteristics.

Missouri River Temperature

Temperature data for the Missouri River was also obtained from the U.S.G.S. for stations at St. Joseph and Hermann, Missouri. Comparison of the St. Joseph and Hermann, Missouri river temperature data with the Lexington measurements shows that a small, relatively consistent temperature profile existed between all three river points. Daily water temperatures did not vary more than 0.5°C among the collected data. Figure 2 shows the distribution of river water temperature over the virus sampling

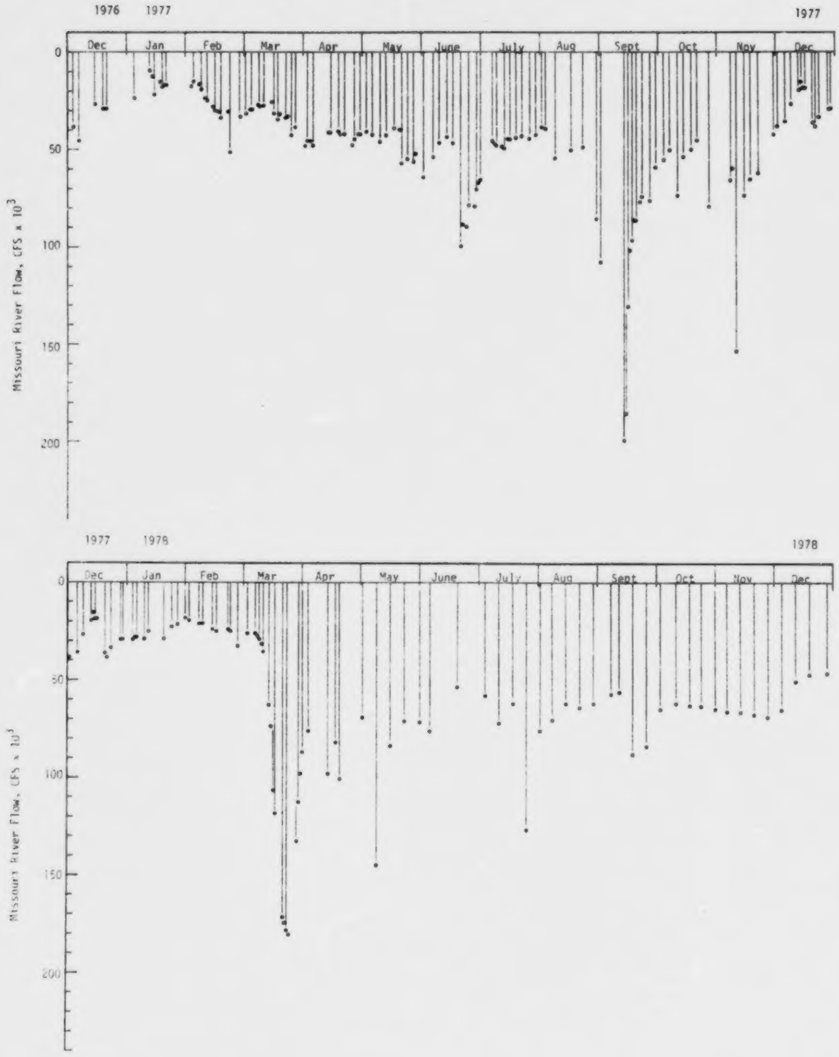

Figure 1 Flow in the Missouri River Corresponding to Virus Sampling Dates, December 1976 through December 1978

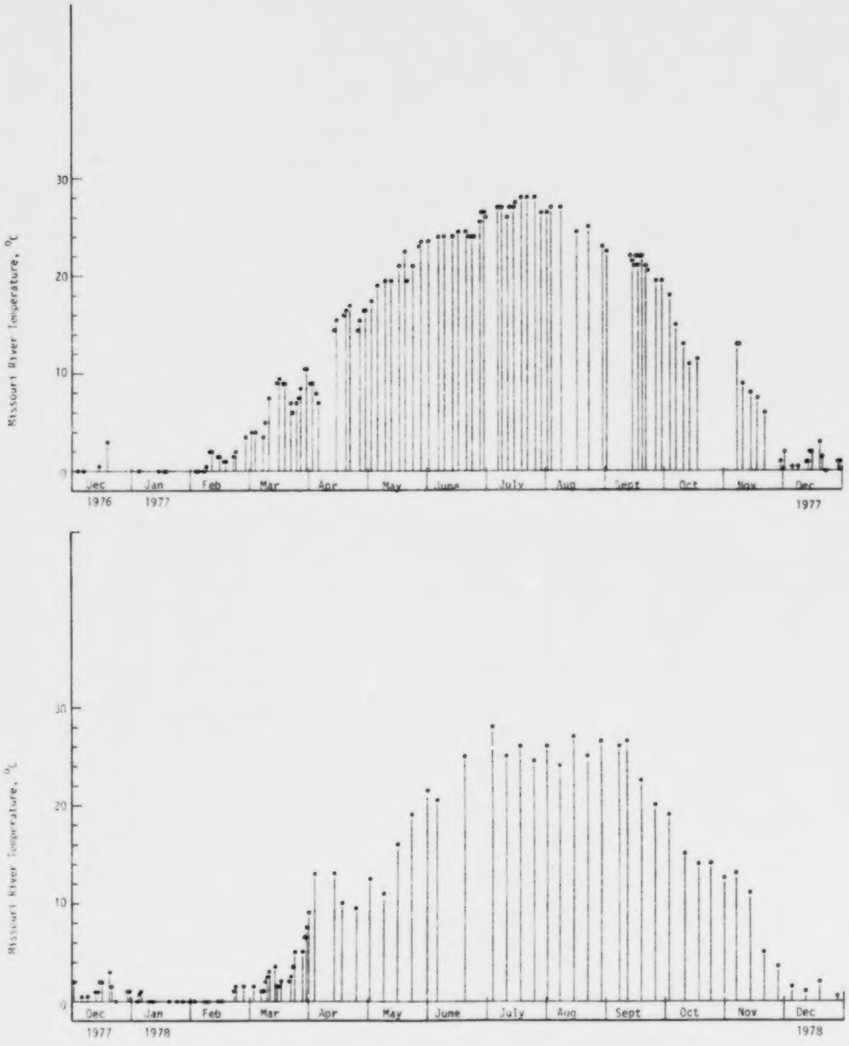

Figure 2 Temperature in the Missouri River Corresponding to Virus Sampling Dates, December 1976 through December 1978

period. The data exhibit a conventional seasonal temperature distribution pattern with the mean peak temperature occurring in the midsummer months of July. Low winter temperatures averaging 0.5°C increased during the spring months to a summer high temperature of 27°C.

Missouri River Turbidity

Figure 3 presents the turbidity levels in the Missouri River measured at Lexington. Similar to the preceeding physical parameters, turbidity measurements exhibit low winter values (less than 10 NTU's) which increase through the spring and reach peak values during the summer. The turbidity data shows an erratic pattern, particularly during the summer months. Comparison of Figures 1 and 3 shows that peak turbidities correspond to rapid increases in river flow (see Figure 1: June, September and November of 1977) resulting from high intensity rain storms. Conversely, a more gradual increase in flow associated with a storm of longer duration (see March 1978) results in an initial decrease in river turbidity. This decrease is followed by a sharp increase which reaches a peak turbidity value similar in magnitude to the high intensity storm water run-offs.

The role of turbidity in the transport of viruses is unclear. A numerical analysis of the virus data will show that approximately 10% of the viruses recovered were directly associated with river water suspended solids from three to five µm in size.

Chemical Parameters

The results of alkalinity and hardness measurements of Missouri River water are presented in Figures 4 and 5, respectively. Contrary to the physical parameters, alkalinity and hardness show high winter, decreasing spring and low summer values. Comparison of Figures 4 and 5 shows that the time traces of both plots are almost identical. Alkalinity values are consistently below those of hardness by 50 to 100 milligrams per liter as calcium carbonate equivalent. Winter highs and summer lows averaged 190 mg/l and 120 mg/l for alkalinity as compared to 270 mg/l and 160 mg/l for hardness.

Although nominally affected by water temperature and turbidity, both alkalinity and hardness levels in the Missouri River are strongly influenced by dilution resulting from the regulated flow scheme and storm water run-off. Note particularly that the largest decreases shown in Figures 4 and 5 correspond to the high storm-water-induced river flows in June 1977, September 1978 and March 1978, shown in Figure 1.

Microbiological Parameters

Total coliform, fecal coliform, standard plate count and yeast organisms were cultured and enumerated in conjunction with each virus sampling. The data for these bacterial parameters are plotted in Figures 6, 7 and 8.

Total and fecal coliform data, presented in Figure 6, exhibit little distinct variation with seasonal changes. Close examination reveals slightly increasing total and fecal coliform counts during the period from early summer through early winter. The fecal fraction generally averages 10% to 15% of the total coliform count. Major peaks in the fecal coliform levels closely follow increases in the total coliform concentra-

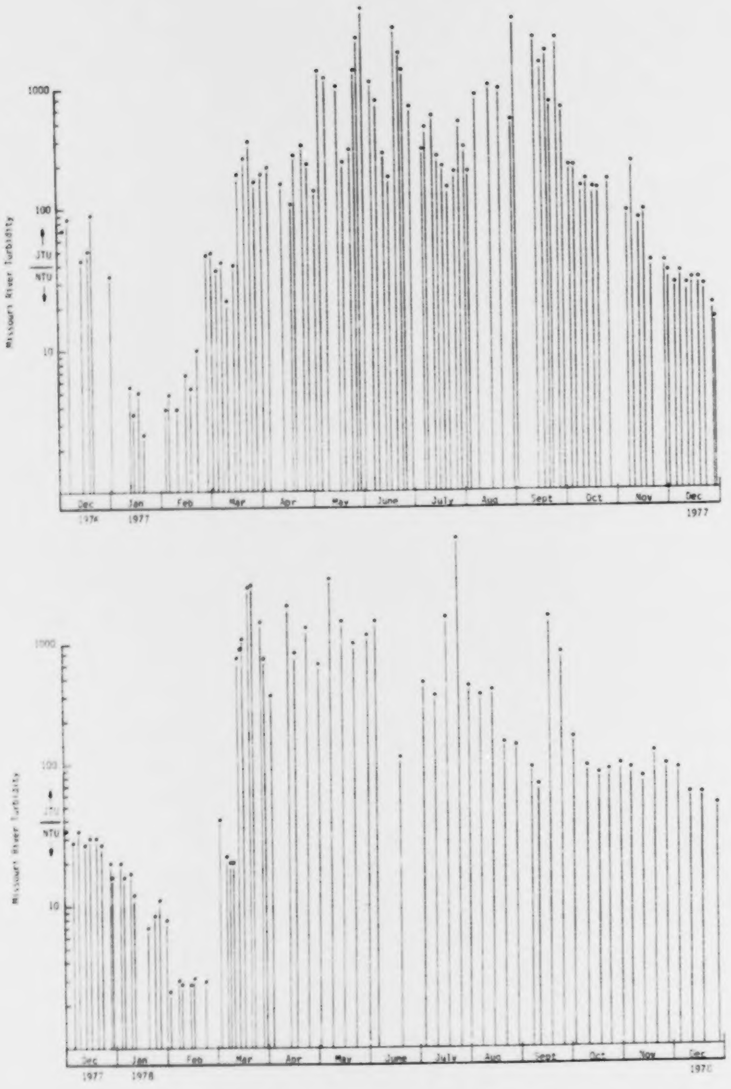

Figure 3 Turbidity of Raw Missouri River Water Corresponding to Virus Sampling Dates, December 1976 through December 1978

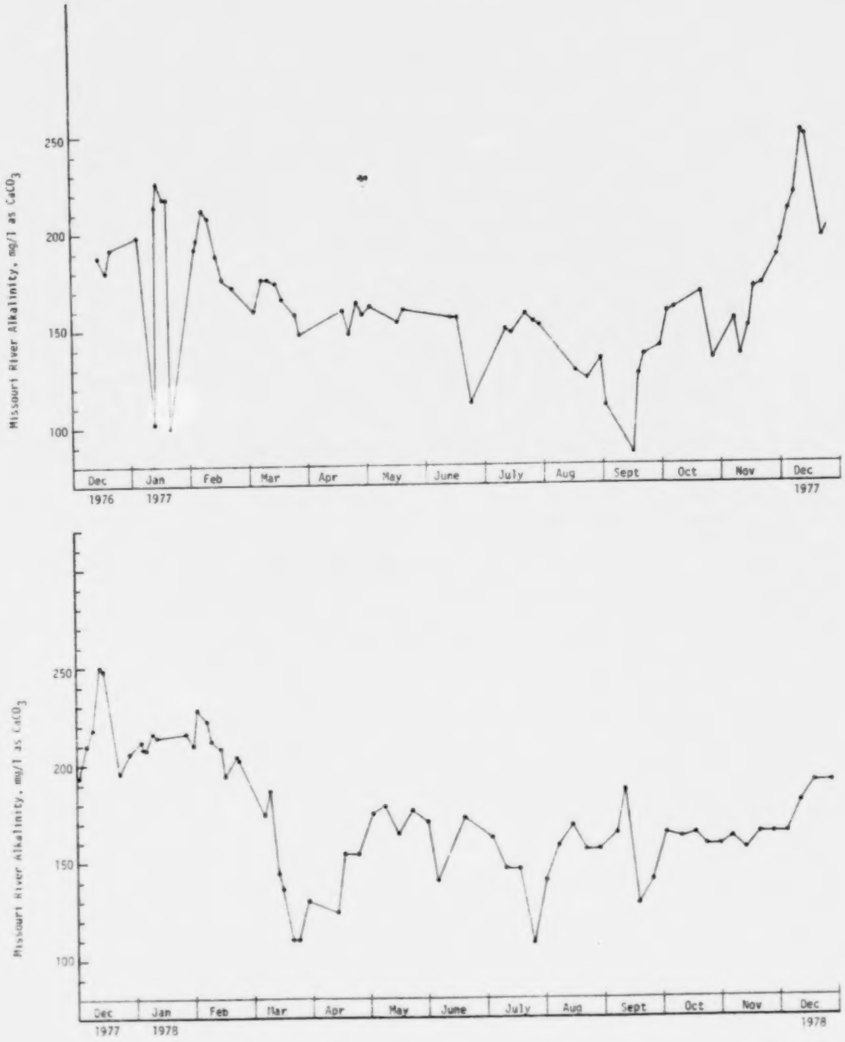

Figure 4 Alkalinity of Raw Missouri River Water Corresponding to Virus Sampling Dates, December 1976 through December 1978

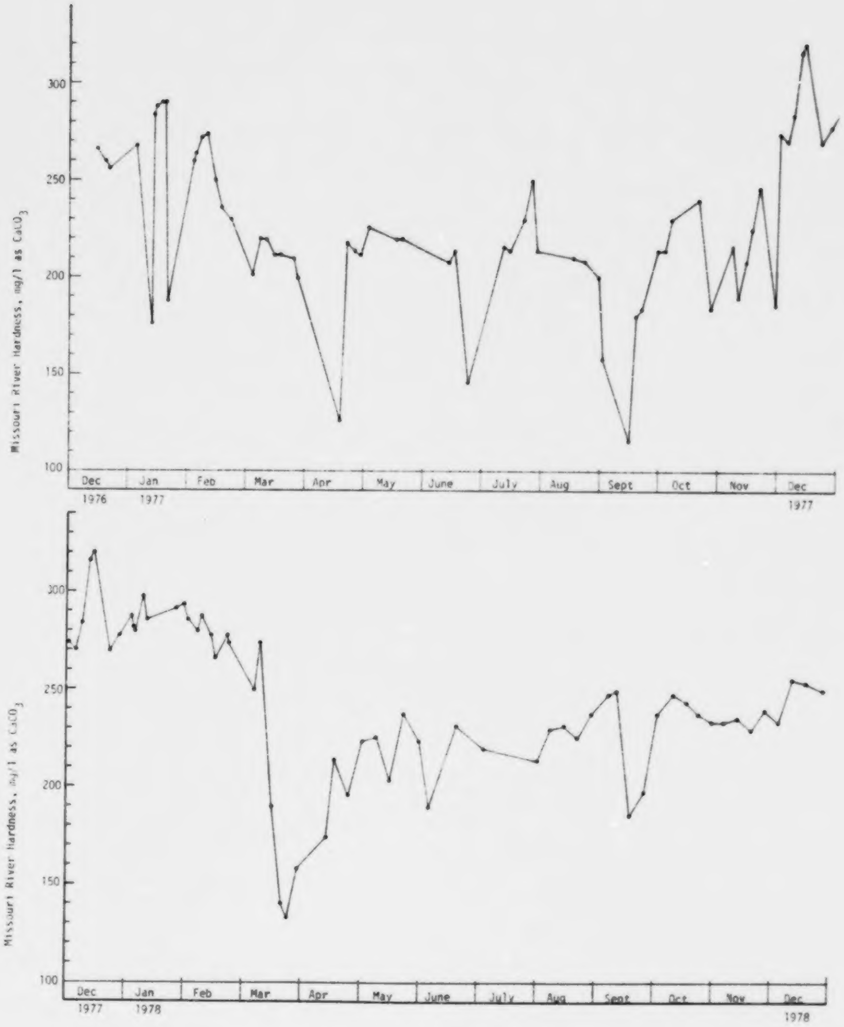

Figure 5 Hardness of Raw Missouri River Water Corresponding to Virus Sampling Dates, December 1976 through December 1978

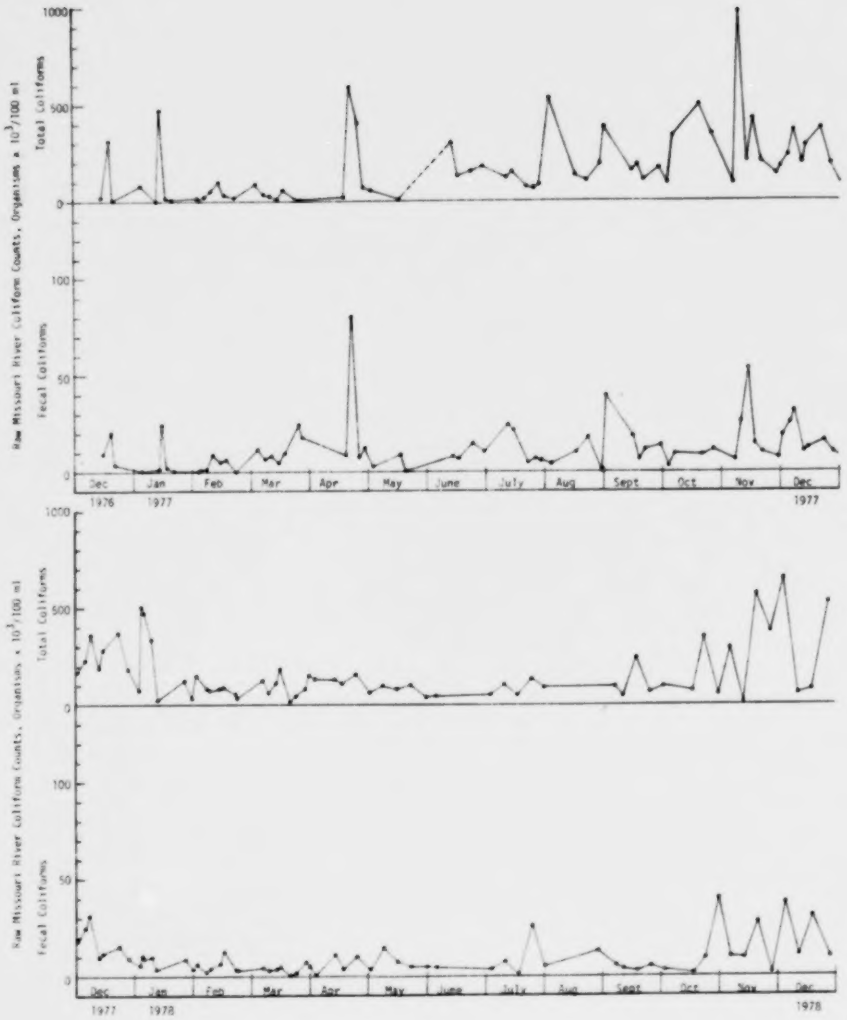

Figure 6 Coliform Counts for Raw Missouri River Water Corresponding to Virus Sampling Dates, December 1976 through December, 1978

tions.

Coliform data comparable to the Lexington results were presented by Hakumat Rai and Hill at the 1978 Anaheim, California WPCF conference (3). In their study on the Missouri River at St. Louis, Mo., Rai and Hill reported a high linear correlation between river flow and fecal coliform densities ($r = +0.85$). However, no specific relationship between the coliform counts and river flow conditions is evident when Figures 1 and 6 are compared. Further analysis using the numerical data associated with Figures 1 and 6 resulted in a Spearman Correlation coefficient "r_s" = +0.32 and a linear correlation coefficient "r" (similar to the Rai-Hill "r") of only +0.19. In addition, total coliform-to-river flow correlations showed an even higher Spearman coefficient ($r_s = +0.37$) whereas Rai and Hill reported "no further direct significance with respect to any of the other bacterial counts."

The difference in the findings between the Lexington, Missouri river study and the Rai-Hill investigation stress two factors concerning the statistical methods for analyzing data from natural systems. First, since the flow and bacterial data are "comparable", dissimilarities in the results may lie in the method of linear analysis used to calculate the "r" correlation values. Statistical methods require that specific assumptions be made about the nature of the data being analyzed. Using variations of a general linear analysis model will impose different assumptions on the same data set and, consequently, will result in discrepancies between correlation coefficients. Second, although the data parameters are "comparable", they are not equivalent. This factor may indicate that for natural systems as large and complex as the Missouri River, data set significance is limited to a finite region up and downstream from the sampling point. It is likely that both the Lexington and St. Louis, Missouri study results are correct for their areas, but are not interchangeable on a river-wide basis.

Figure 7 presents the results of the standard plate count enumerations. Generally, standard plate counts follow a pattern resembling the physical river water parameters (i.e., low winter - high summer values). The data for the 1977 calendar year show peak counts occur in the summer months of May through September. In 1978, however, increasing plate counts were observed earlier in the spring months of March and April. Contrary to the coliform data of Figure 6, standard plate organisms exhibit a large and distinct variation with seasonal changes. The plate count data shows a repetitive pattern of high and low points. A particularly significant statistical relationship exists between river flow and standard plate count organism levels.

Yeast organisms (Figure 8) are important in the environmental cycle because of their ability to metabolize complex carbohydrates. The significance of yeasts as a water quality parameter, however, is limited by the comparatively low numbers of these organisms found in natural waters.

Virus Data - "General"

Analyses of the virus data accumulated between December 1976 and December, 1978, are presented in Tables 1, 2 and Figures 9 through 27. As before, all data shown in this section pertains to raw Missouri River water samples only.

A general numerical analysis of the virus data is listed in Table 1. Out of 216 samples collected during the period 12/13/76 to 12/27/78, a total of 545 viruses were

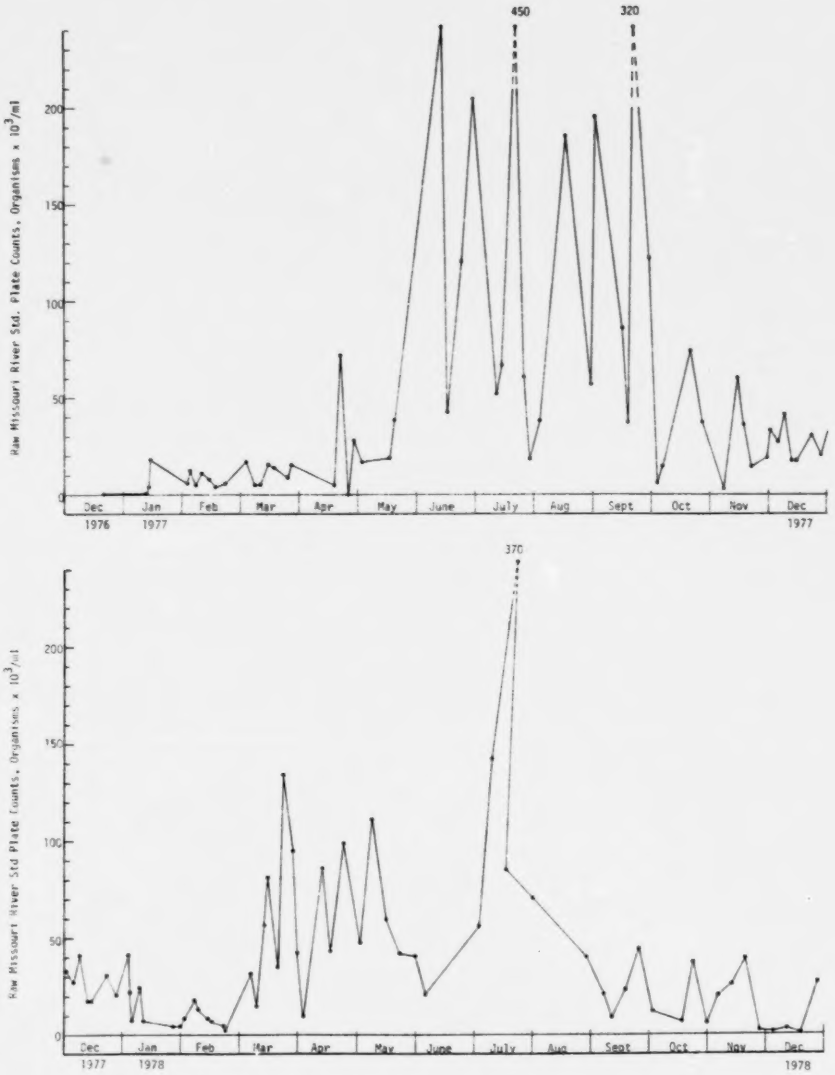

Figure 7 Standard Plate Counts for Raw Missouri River Water Corresponding to Virus Sampling Dates, December 1976 through December 1978

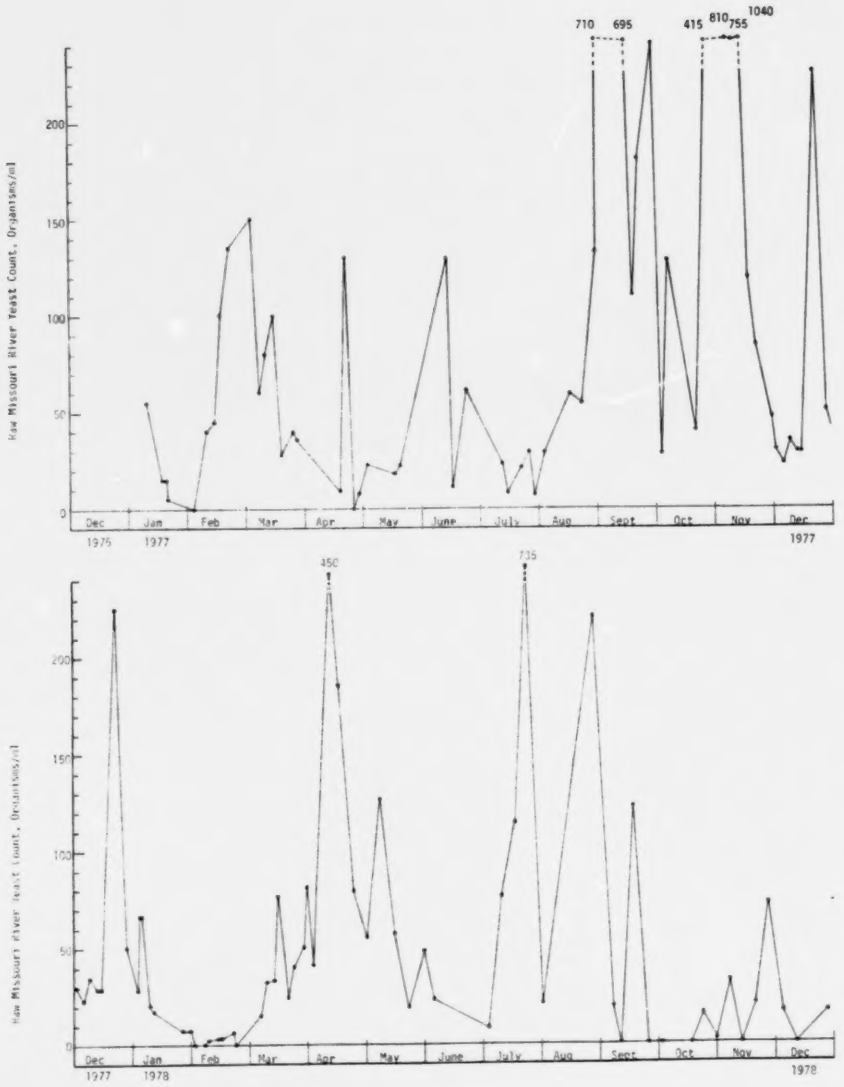

Figure 8 Yeast Organism Counts for Raw Missouri River Water Corresponding to Virus Sampling Dates, December 1978 through December 1978

TABLE 1. ANALYSIS OF RAW MISSOURI RIVER WATER VIRUS DATA

Item	Amount
Total Number of Raw Water Samples (12/13/76 thru 12/27/78)	216
*Total Polioviruses Recovered	375
Total Coxsackie Viruses Recovered	58
Total ECHO Viruses Recovered	29
Total Unidentified Viruses	83
Total Virus Count (12/14/76 - 5/1/78)	545
% Poliovirus of Total Virus Count	68.8
% Poliovirus of all identified viruses	81.2
% Coxsackie of Total Virus Count	10.6
% Coxsackie of all identified viruses	12.6
% ECHO Virus of Total Virus Count	5.3
% ECHO virus of all identified viruses	6.3
Number of Viruses Recovered on Prefilter	53
% of Total Virus Recovered on Prefilter	9.7%
Number of Viruses Recovered by Adsorption from Prefilter Water	492
% of Total Virus Recovered by Adsorption from Prefilter Water	90.3%

*Poliovirus Type I	76%	Polio I of Total Polio	20.3%
Poliovirus Type II	197%	Polio II of Total Polio	52.5%
Poliovirus Type III	102%	Polio III of Total Polio	27.3%

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recovered (an average of 2.5 viruses per sample). Of this total, polio, Coxsackie and ECHO viruses constituted 68.8%, 10.6% and 5.3% of the virus count, respectively. The remaining 15.2% of the virus isolates recovered were unidentified. The majority of these unidentified isolates resulted from serum neutralization tests indicating that cultured plaques consisted of multiple virus types. Viruses showing mixed serum neutralization tests are generally considered to be of the polio group.

In addition to the breakdown of the recovered enteroviruses into groups and types, Table 1 lists the total quantities of viruses associated with the river water suspended solids greater than 3.0 um. River solids removed on the Aquella "prefilter" were backwashed (eluted) and assayed following the same procedures used to recover enteroviruses retained on the pleated "virus absorber".

As shown in Table 1, approximately 10% of the total number of virus isolates were directly associated with the Missouri River suspended solids. Several types of viruses from each of the three enteric groups were eluted from the prefilter concentrates. Polioviruses were the most predominant group with 32 identifications out of 53 recoveries. The 32 isolates were split relatively evenly between the three polio serotypes. Coxsackie and ECHO viruses were recovered in smaller quantities of 8 PFU and 6 PFU, respectively. Of the Coxsackie group, types B3, B4 and B5 were identified. All but one of the ECHO group were type 7. The single other type was identified as ECHO-1.

Table 2 compares the virus recovered on the prefilter with the turbidity of the raw Missouri River water. Comparison of the number of viruses recovered from the prefilter versus that recovered from the absorber shows that the overall 10% figure is not constant. The net 10% solids associations value is a result of the fact that in the majority of cases where the prefilter was used, no viruses were recovered from the entrapped river sediments.

Virus Data "Time Sequence"

Expanded versions of the data summarized in Table 1 are presented in Figures 9, 10 and 11. In these analyses a sample volume of 0.38 m^3 (100 gallons) was selected as the standard and all of the virus data were normalized to this volume. The standardized raw water virus concentrations were then plotted on a time sequence abscissa similar to Figures 1 through 8.

Figure 9 shows the distribution of all 545 virus isolates. Although some anomalies exist, Figure 9 shows peak virus concentrations occurring in the 1976-1977 winter and spring seasons followed by relatively low concentrations in the summer and fall. Higher virus concentrations again appear in the spring and winter months of 1978. Virus recoveries averaged 10 PFU/100 gal. in the winter-spring period of 1976-1977 and 1.5 PFU/100 gal. in the following summer-fall season. However, the 1977-1978 winter-spring recovery period showed virus concentrations to average only one half that of the previous winter-spring season or 5 PFU/100 gal.

Following the sequence of Table 1, Figure 10 plots the distribution of total poliovirus. The seasonal virus concentrations of Figure 10 are very similar to those of Figure 9. Polioviruses exhibit higher concentrations in the winter months of 1976-77 and again in 1977-78.

Correlations between average recovery magnitudes for the successive winter periods were more consistent for the poliovirus data. Average poliovirus concentrations were 7 PFU/100 gal. and 4 PFU/100 gal. during peak recovery periods (Figure 10) as compared to the 10 PFU/100 gal. and 5 PFU/100 gal. averages for the total virus data (Figure 9). While several sporadic poliovirus recoveries were made in the 1977 summer season, the concentrations did not exceed 4 PFU/100 gal. and the majority of samples yielded zero polioviruses.

Closer inspection of Figure 10 shows a bi-modal distribution for the peak winter season virus concentrations. The peak concentrations of December 1976 and January 1977 are followed by an apparent decline in February and a second peak in March. This bi-modal pattern repeats itself in the December 1977 through March 1978 period but at an overall lower magnitude in the virus concentration.

Figure 11 is a graphical representation of the Coxsackie and ECHO virus data from Table 1. Added together, Coxsackie and ECHO virus recoveries amount to less than 20% of the total. The Figure 11 data indicates that the seasonal distribution of these viruses is relatively constant throughout the sampling period.

Generally, Coxsackie and ECHO virus concentrations range between 1 and 3 PFU/100 gallons. However, several large sample recoveries occurred in January and June 1977. The three most extreme concentrations, all of the Coxsackie group, were 16, 8 and 8 PFU/100 gallons. These values are comparable to the average recoveries for the poliovirus data of Figure 10.

Modified Virus Data - "Moving Average"

Collectively, Figures 9, 10 and 11 exhibit many "zero" virus recovery dates. Many of these zero values appear directly before and after peak virus recoveries. To further assess the relevance of the zero recoveries together with the concentration peaks, a statistical moving average "smoothing technique" was applied to the data.

The moving average consists of summing three consecutive virus recovery values and dividing the summation by three to obtain an arithmetical average. This average is then plotted at the centroid of the time period covering the consecutive sampling data.

The moving average analyses for the total and polio virus data are presented in Figures 12 and 13. Compared to their corresponding "raw" data plots (i.e., Figures 9 and 10), the moving average results show several important trends. First, a much more distinctive seasonal variation in virus concentrations is exhibited. Second, virus recovery magnitudes between successive spring periods show increased consistency. Third, the previous bi-modal distribution for the winter-spring peak virus concentrations now appear to be multi-modal in nature.

Closer examination of the multi-modal distribution in Figure 11 (aside from the June 1977 Coxsackievirus recoveries) reveals a damped harmonic pattern, i.e., a sequence of peak values each of generally lower magnitude than the previous peak. The damped response pattern started in January 1977. Recovery peaks diminished through the spring becoming a succession of equivalent concentration peaks during the summer-fall months. The sharply increasing November 1977 concentrations seemed a prelude to a repeated pattern. However, the December-January virus recoveries did not attain the levels of the previous season.

TABLE 2. VIRUS RECOVERY ON PREFILTER

Turbidity (NTU)	Total Viruses Recovered (PFU/100 gal)	Viruses Recovered on Prefilter* (PFU/100 gal)	% of Total Virus on Prefilter
22	5	1	20
38	21	4	19
40	3	1	33
41	6	1	17
44	12	5	42
49	8	6	75
60	11	6	55
63	2	2	100
66	11	2	18
70	17	10	59
70	2	2	100
75	18	2	11
80	2	1	50
80	4	2	50
90	2	2	100
105	8	1	13
120	2	2	100
125	4	4	100
130	4	2	50
136	1	1	100
190	2	2	100
200	3	3	100
200	10	3	30
**	492	0	0
Total	545	53	10

Note: Data is ranked by increasing turbidity and does not correspond to any specific time sequence.

* 3.0 - 5.0 μ cartridge prefilter

** Summation of all samples where no viruses were recovered from prefilter sediments.

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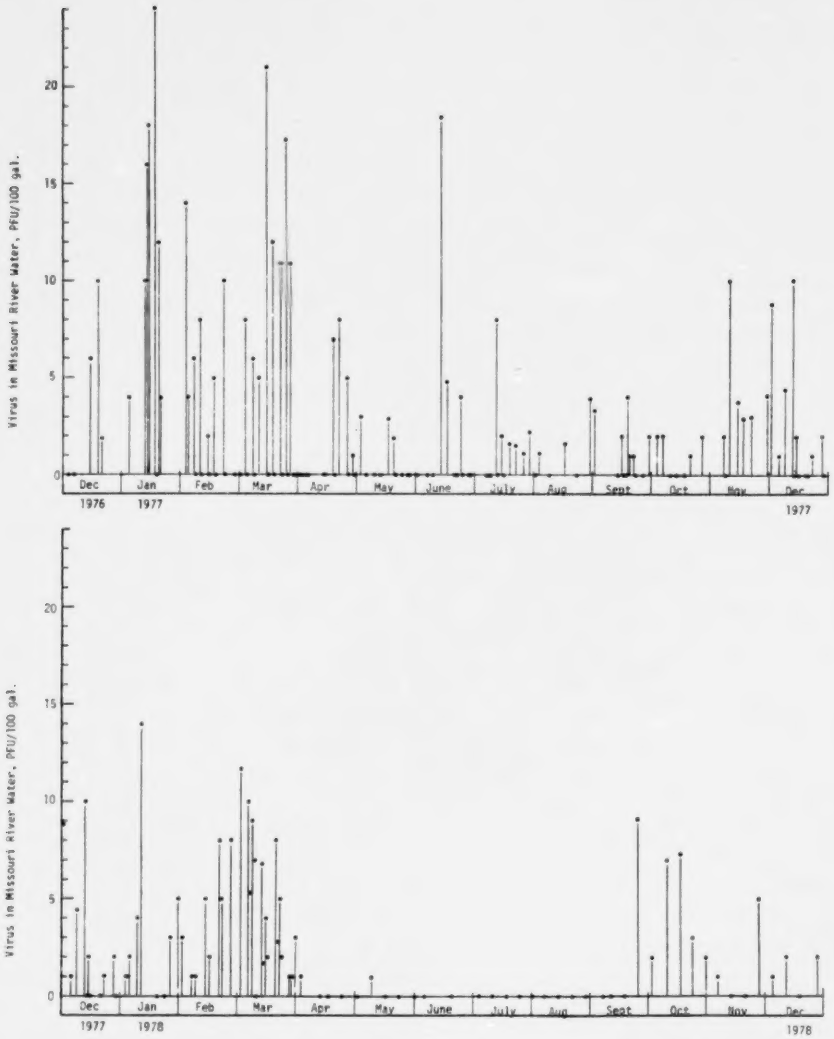

Figure 9 Virus in Raw Missouri River Water, December 1976 through December 1978

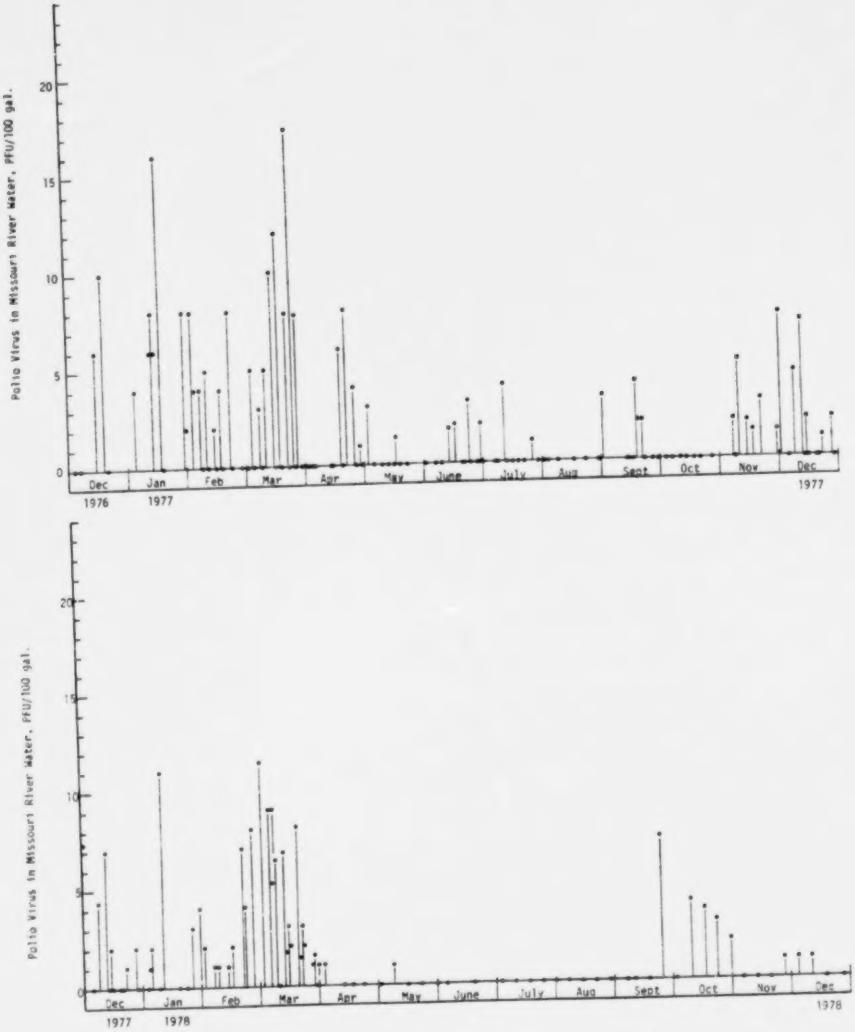

Figure 10 Poliovirus in Raw Missouri River Water December 1976 through December 1978

The poliovirus moving average of Figure 13 exhibits a harmonic response pattern similar to Figure 12. Note that without the June Coxsackievirus anomalies, the damped pattern is more consistent throughout the sampling period.

Modified Virus Data - "Virus Flow"

All the virus data results of 9 through 13 have been presented with ordinate axis units of virus concentration, i.e., number of viruses recovered per 0.38 m^3 (100 gallons) of raw Missouri River water. An equally important method of data analysis is to present the time sequence samples based on the total number of viruses in the Missouri River flowing past Lexington, Mo. Essentially, this total virus number is the virus concentration data adjusted for the seasonal flow variations in the Missouri River.

Adjustment for river flow requires the assumption that the observed virus concentrations were homogeneous throughout the Missouri River at Lexington, Missouri. Based on this assumption virus concentrations were multiplied by the average daily river flow corresponding to their sampling dates. The resulting units of "virus per second" have led to the use of the term "virus flow".

Figures 14 and 16 present the virus flows in the Missouri River corresponding to the total virus and poliovirus results of Figures 9 and 10. Figures 15 and 17 show the virus flow data further modified by the moving average smoothing technique similar to Figures 12 and 13. Comparison of Figures 12 and 15 shows the relative seasonal variation in the virus flow to be much less than the seasonal variation of the virus concentration. Figure 15 also shows a multi-modal seasonal recovery distribution. Contrary to the virus concentration results, the multi-modal virus flow distribution does not show a damped harmonic response pattern. Additionally, the highest virus recovery period has shifted from the winter-spring of 1977 to the winter-spring of 1978. Figure 12 (virus concentration) shows the greatest of the winter-spring recovery periods occurred in January, 1977 whereas Figure 15 (virus flow) indicates the largest virus population occurred in November, 1977 and March 1978.

The poliovirus flow seasonal distribution (Figure 17) does not follow distribution of the total virus flow as closely as might be expected from comparison of the virus concentration graphs (Figures 12 and 13).

Virus Indicator Correlations

Individual plots for physical, chemical, microbiological and viral parameters were presented in the previous sections. To determine the applicability of these parameters as virus population indicators, the graphs shown in Figures 18 through 28 were plotted. Each of the figures plots virus concentration versus one of the measured physical, chemical or microbiological parameters. Evaluation of these parameters as possible virus population indicators follows the data analysis sequence of Figures 1 through 9 (i.e., physical parameters: flow, temperature, etc.) Note that circular points indicate single data observations while squares indicate two or more concurrent data observations.

Qualitative examination of the figures indicates that no exceptionally well-defined relationships exist between the data. In fact, all physical and microbiological parameters appear to be inversely proportional to observed virus concentrations. Only the two chemical parameters, alkalinity and hardness, seem to show positive relations

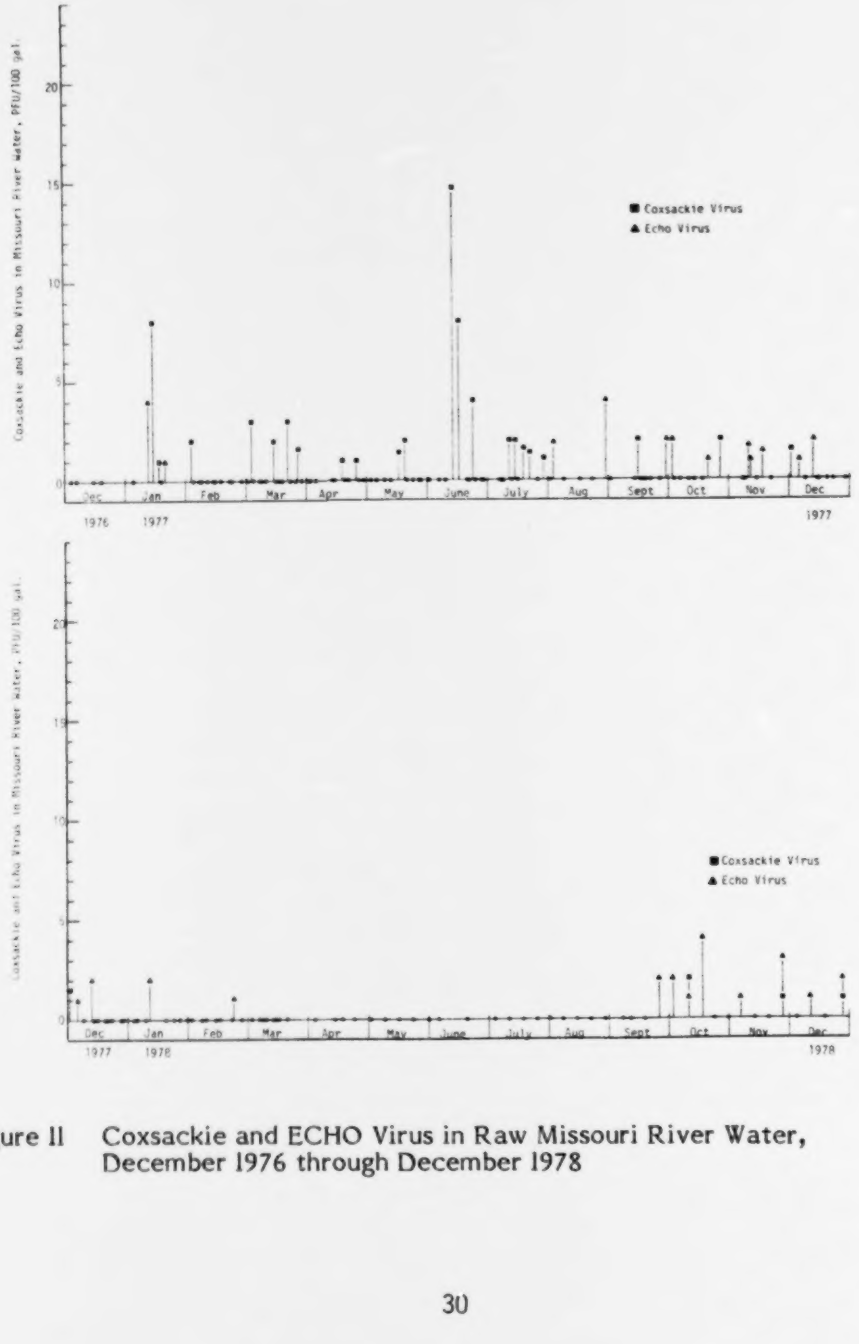

Figure 11 Coxsackie and ECHO Virus in Raw Missouri River Water, December 1976 through December 1978

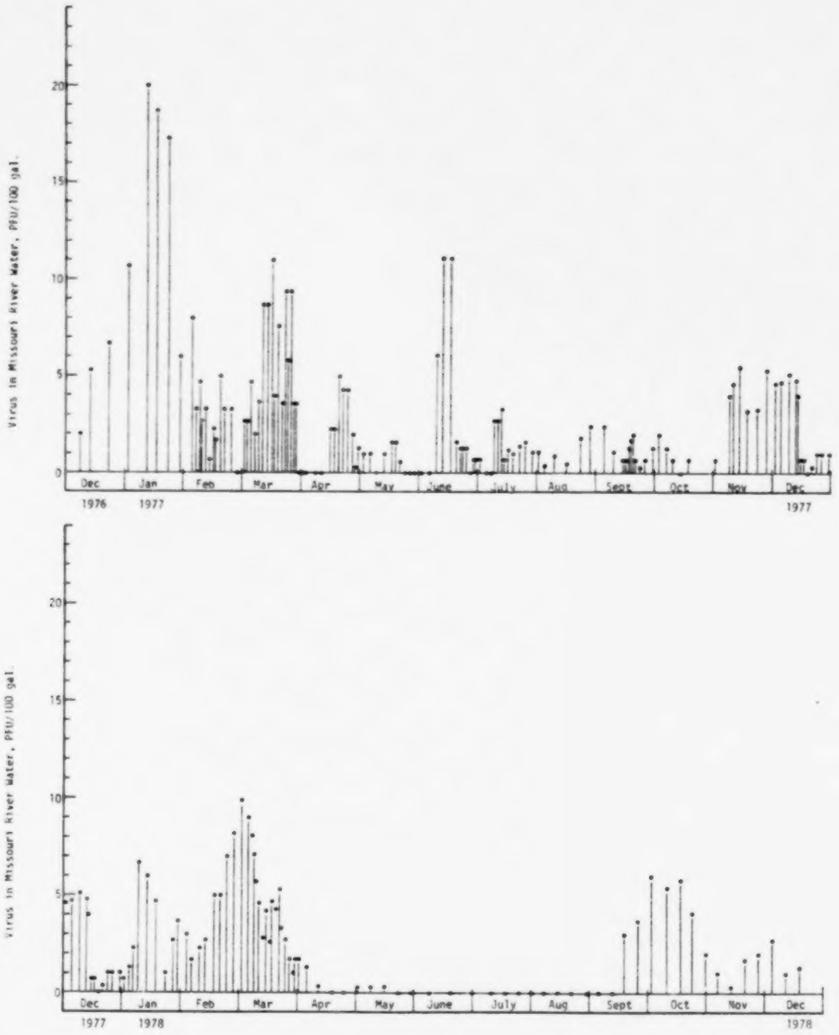

Figure 12 Virus in Raw Missouri River Water, Moving Average, December 1976 through December 1978

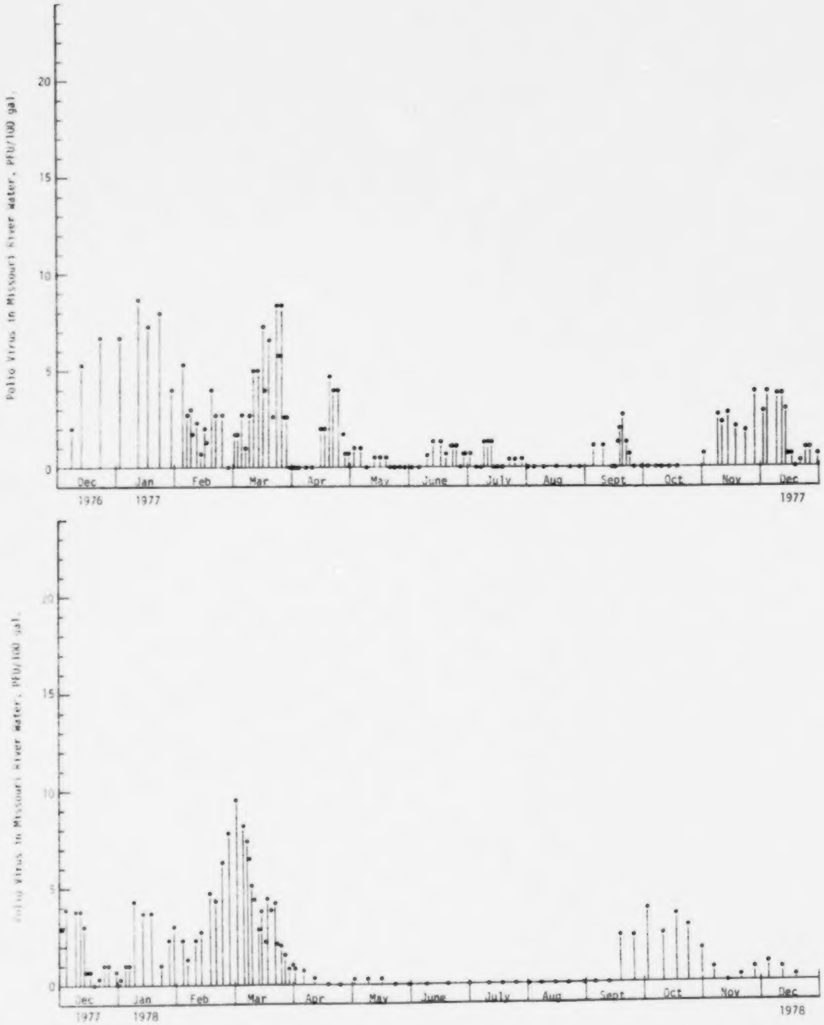

Figure 13 Polio Virus in Raw Missouri River Water, Moving Average, December 1976 through December 1978

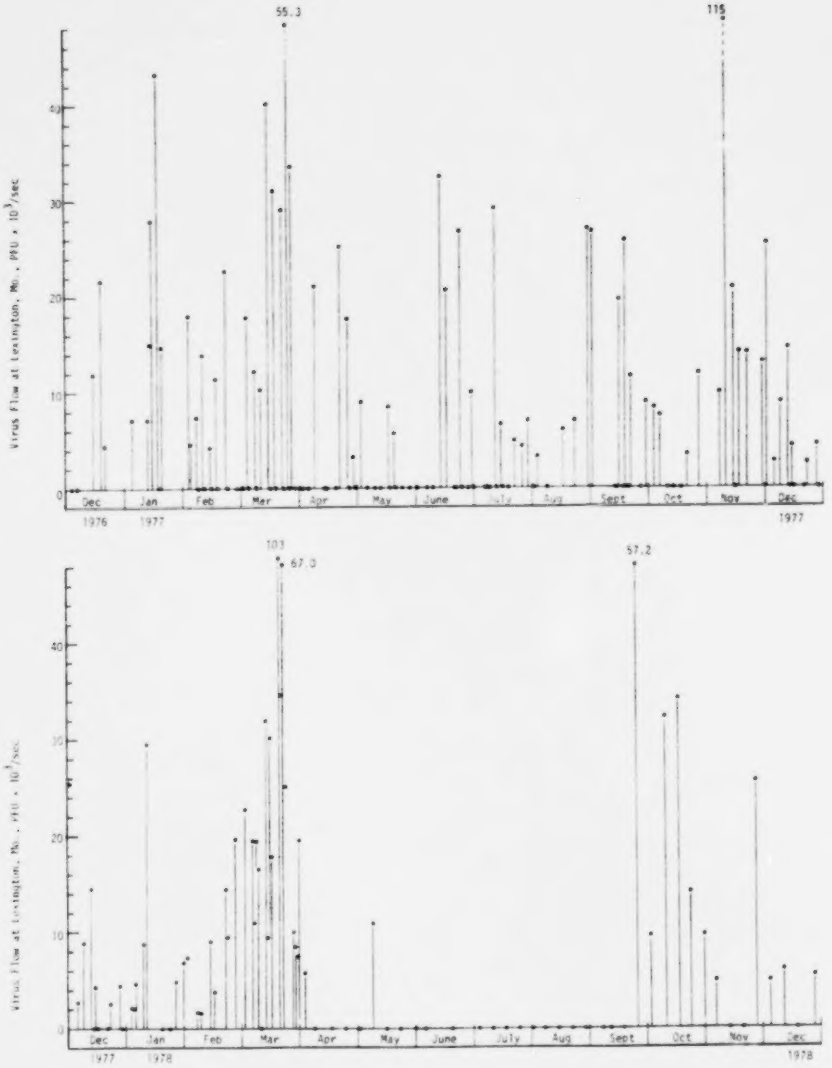

Figure 14 Virus Flow in Raw Missouri River Water at Lexington, Missouri December 1976 through December 1978

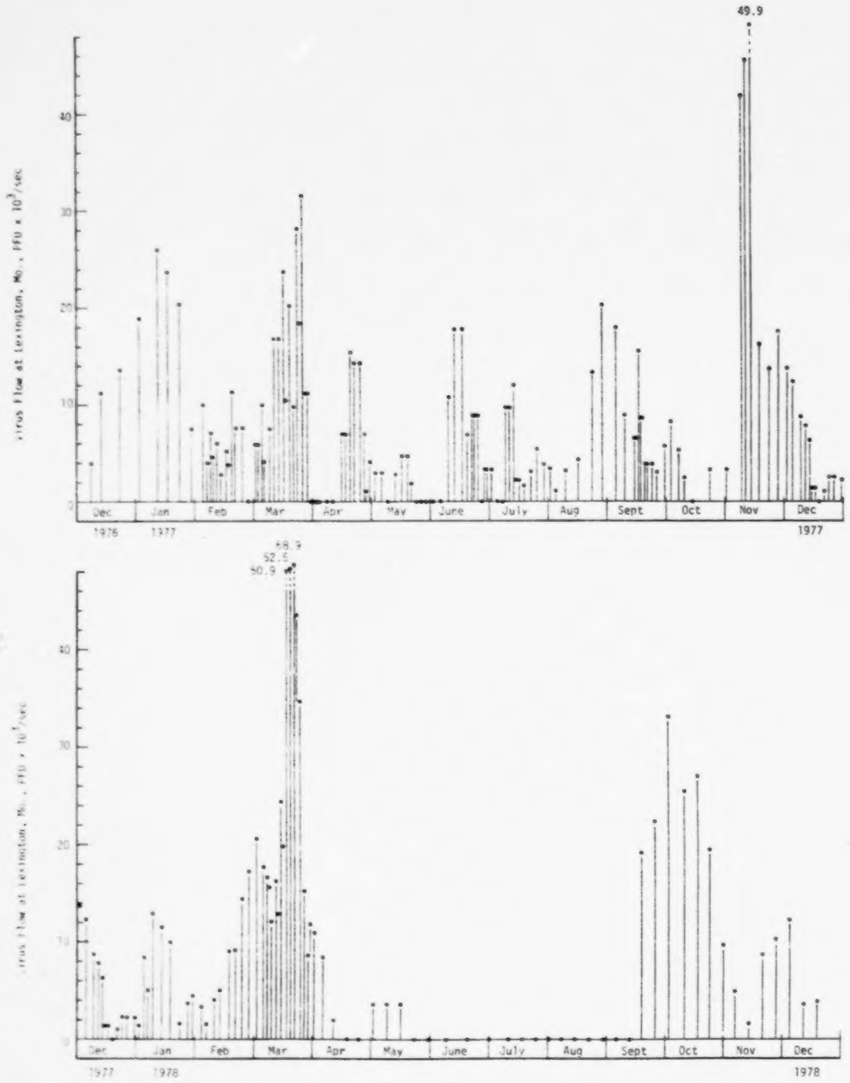

Figure 15 Virus Flow in Raw Missouri River Water, Moving Average, at Lexington, Missouri, December 1976 through December 1978

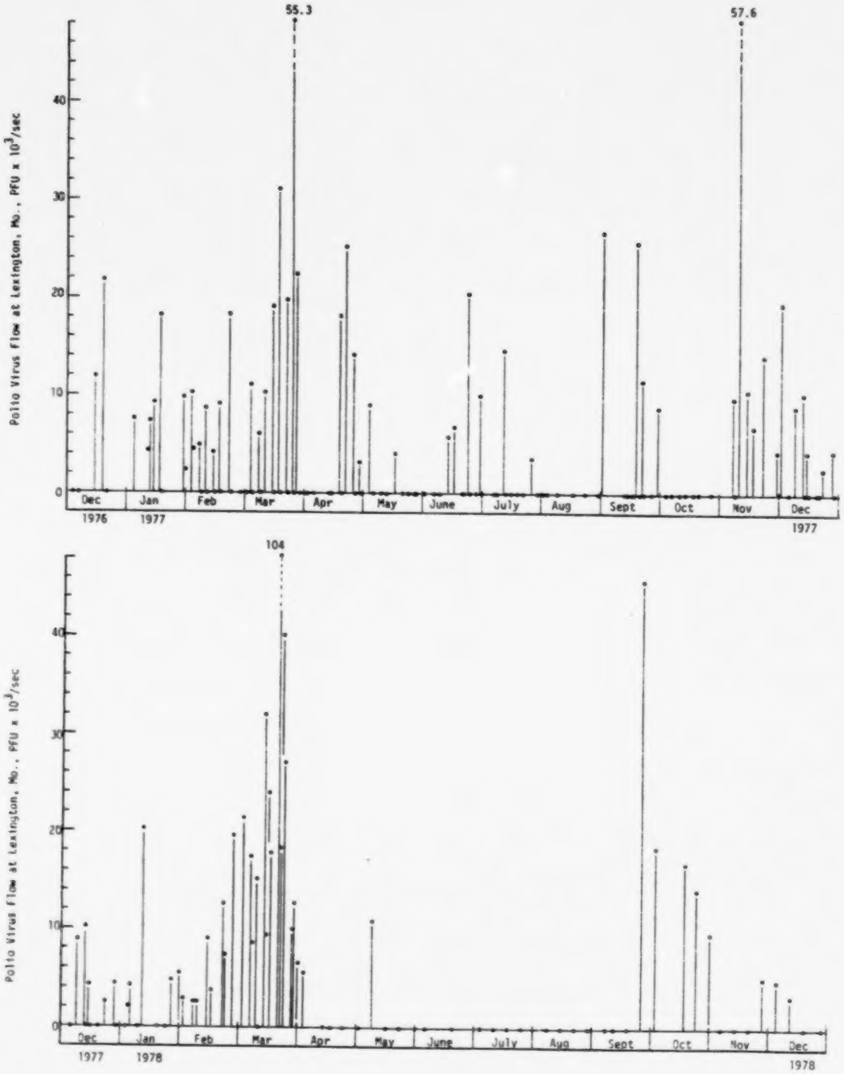

Figure 16 Polio Virus Flow in Raw Missouri River Water at Lexington, Missouri, December 1976 through December 1978

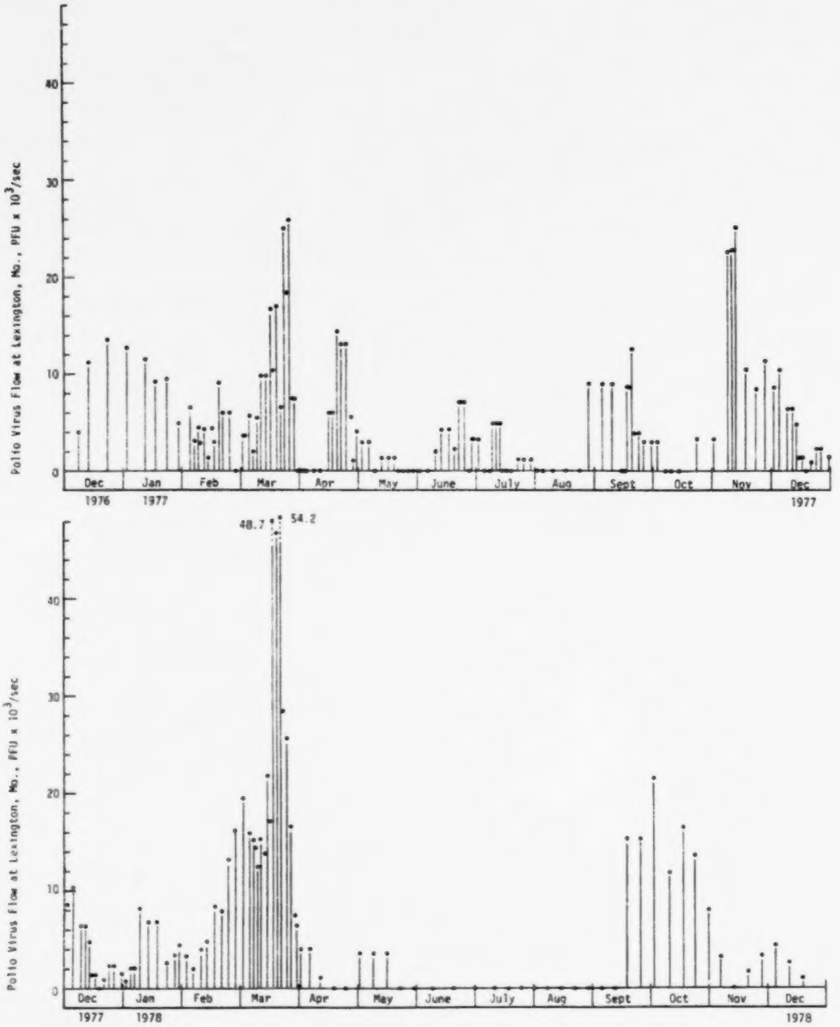

Figure 17 Polio Virus Flow in Raw Missouri River Water, Moving Average at Lexington, Missouri, December 1976 through December 1978

with the virus concentrations. To further quantify these observations, simple linear regression and Spearman Rank Correlation analyses were performed on the data.

The linear regression analysis calculates the coefficient"r^2" which measures the amount of variation in the virus concentration data that can be attributed to a particular indicator parameter. For example: if all seasonal variations in the virus data were directly and linearly proportional to the turbidity levels in the Missouri River, then $r = +1.0$. In another manner of speaking, linear regression analysis calculates the best fitting straight line for the given data and then indicates how representative of the data that line is in terms of the "r^2" coefficient.

From a slightly less quantitative standpoint, the Spearman Rank analysis calculates "r_s" which measures the tendency of high values in the virus concentration data to be associated with high values in the indicator parameter data or vice versa. Effectively, the Spearman statistical analysis attempts to answer the following questions:

1) Are the data sets mutually independent?
   ($r_s = 0.0$)

2) Are the data sets mutually dependent?
   ($r_s = +1.0$ or -1.0)

3) How often do large values of one data set tend to be paired with large values of the other data set?
   ($r_s = 0.0$ to $+1.0$)

4) How often do large values of one data set tend to be paired with small values of the other data set?
   ($r_s = 0.0$ to -1.0)

For example, if the virus concentrations and river water temperatures were perfectly inversely related (i.e., high virus counts were consistently recovered at low river temperatures and low virus counts were consistently recovered at high river temperatures) then $r_s = -1.0$.

It is important to note that the Spearman correlation only calculates the r_s coefficient based on the magnitudes of the data pairs. The linear regression analysis, however, calculates "r^2" by presupposing that the data has been obtained with negligible error in its measurement and that it is normally distributed. Previous results indicated that the data obtained from the Lexington, Mo. study may not meet the linear regression analysis boundary conditions.

The results of the linear regression and Spearman ranking analyses are presented in Tables 3 and 4. Table 3 lists the correlation coefficients associated with Figures 18 through 27. Table 4 lists the coefficients for additional correlations between the physical, chemical and microbiological parameters themselves.

The negative arithmetic signs for the Spearman coefficients in Table 3 confirm the hypothesis that the physical and microbiological river water parameters are inversely correlated to virus concentration. The positive signs for alkalinity and virus concentrations in raw wastewater are, in fact, highest during the summer and fall months, sewage treatment processes generally function at maximum efficiency during this same period. Consequently, higher virus removals during wastewater treatment should be expected during the summer and fall, partially accounting for the lower river water virus concentrations.

Finally, during warm weather, river flows and hence turbidities for the Missouri River increase substantially. The presence of high suspended solids concentrations may tend to further reduce the efficiency of the virus recovery methods employed.

Distribution of Virus

The analysis of the Missouri River virus data (Table 1) indicates that polioviruses were recovered seven times more frequently than Coxsackie virus and twelve times more frequently than ECHO virus. The far greater recovery of polioviruses is consistent with results obtained by other investigators who have studied enteric virus distributions in wastewater and found polioviruses to be the most abundant species. The seasonal poliovirus distribution (Figure 10) almost duplicates the total virus recovery distribution of Figure 9. Both public health officials and medical researchers proclaim that the overwhelming majority of polioviruses recovered from wastewater or contaminated waters will be of the vaccine strain. This observation raises the following question:

> "Could public immunization programs using live attenuated virus
> vaccines, such as polio vaccine, together with carrier populations be
> responsible for the periods of high virus concentration encountered
> in the Missouri River investigation?"

Data reported by Zdrazideh, et. al., (9) showed poliovirus in raw wastewater to be proportional to the percentage of positive stool samples taken from children vaccinated with live attenuated virus. Bagdasar'yan (10) reported results similar to those of Zdrazideh on two separate occasions. T-marker analysis on the Missouri River polio isolates indicated that all cultures tested, except for two, were vaccine strain polio viruses. However, aside from the encouraging background data, attempts to correlate Missouri State Health Department vaccination records with the Missouri River poliovirus data provided no supportive evidence.

Contrary to the seasonally variable poliovirus distribution, the relatively constant Coxsackie and ECHO virus concentrations would indicate that a small amount of viral disease is continuously circulating throughout the population. It is possible that the continuous low-level appearance of the Coxsackie and ECHO viruses can be attributed to sub-clinical illness such as nonbacterial gastroenteritus.

As pointed out in the results section, the Coxsackievirus concentration showed a substantial increase during June 1977. There is a reasonable possibility that the observed increase is a result of water sport activities (immediately following the start of summer vacation for school children) establishing the classic fecal-oral route of enterovirus infection. Investigations by Kelly, et. al., (11) Honig, et. al., (12) and Melnick (13) showed similar results in that Coxsackie and ECHO virus concentrations were found to increase periodically and that they ofen were isolated in significantly higher levels than the average poliovirus recoveries. Melnick concluded from his investigations that the distribution of Coxsackie and ECHO viruses, including the

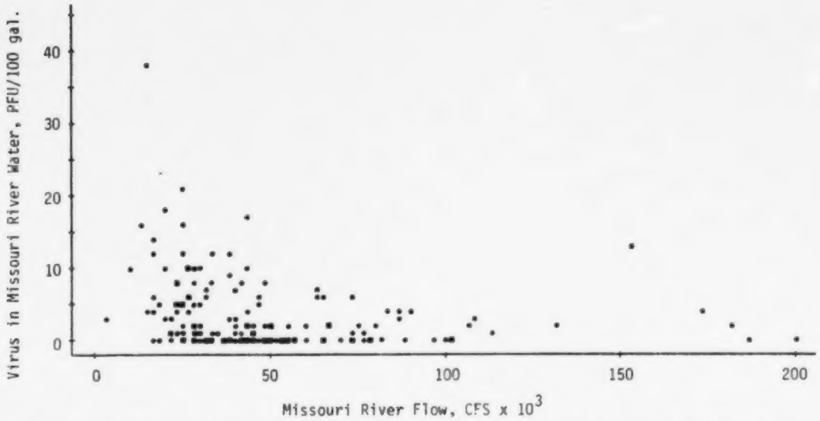

Figure 18 Virus versus River Flow of the Missouri River at Lexington, Missouri

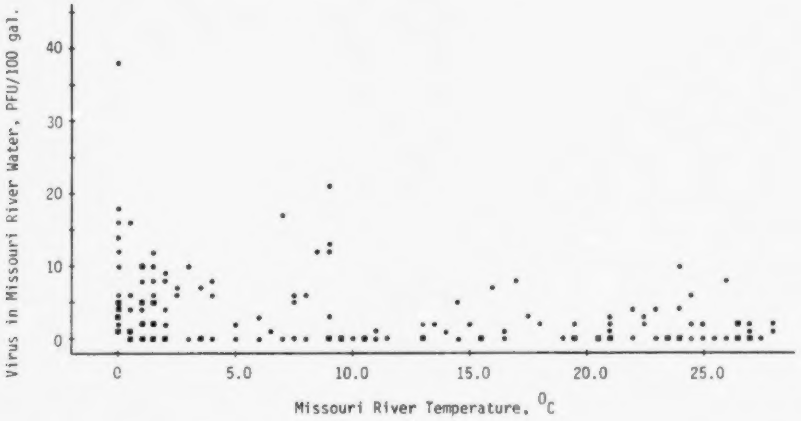

Figure 19 Virus versus Water Temperature of the Missouri River at Lexington, Missouri

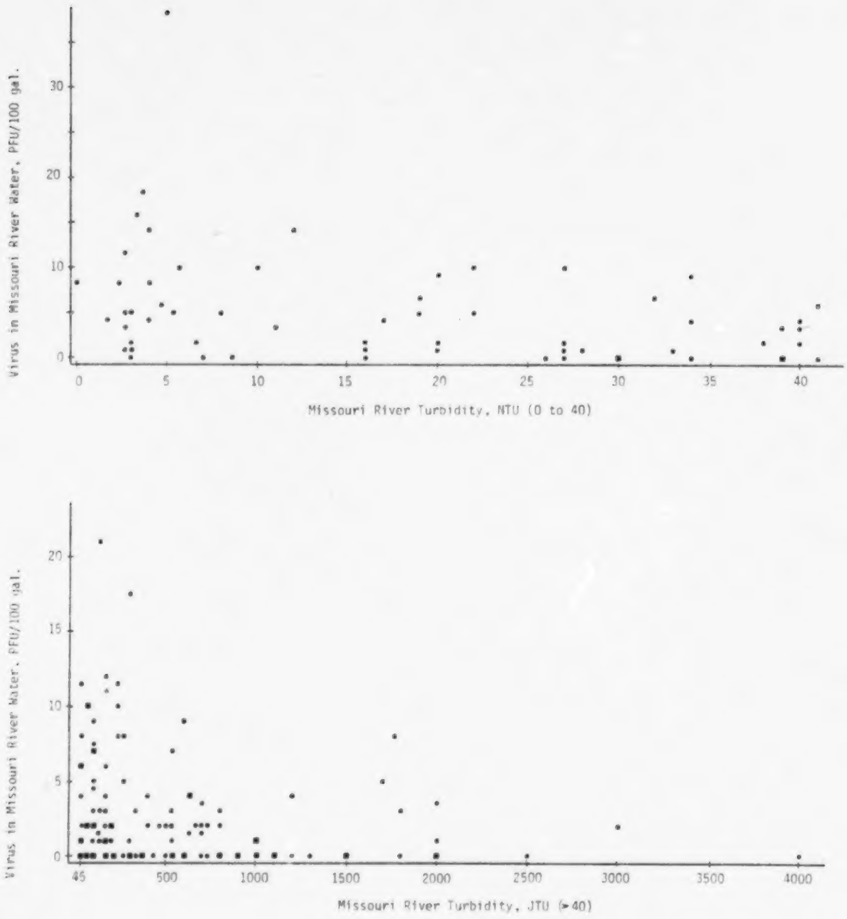

Figure 20 Virus versus Turbidity of the Missouri River at Lexington, Missouri

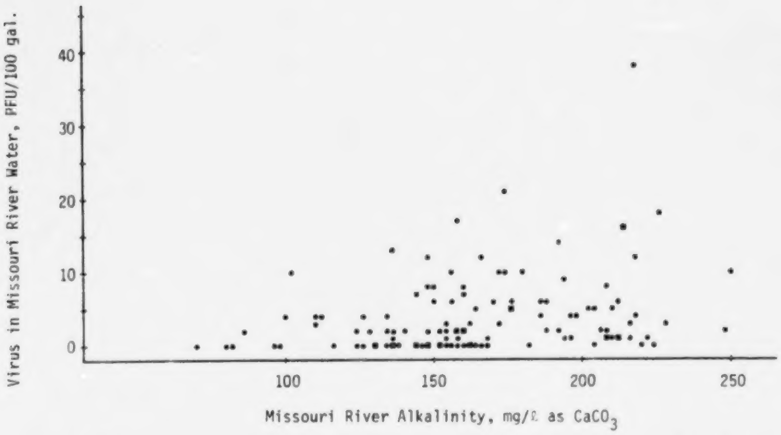

Figure 21 Virus versus Alkalinity of the Missouri River at Lexington, Missouri

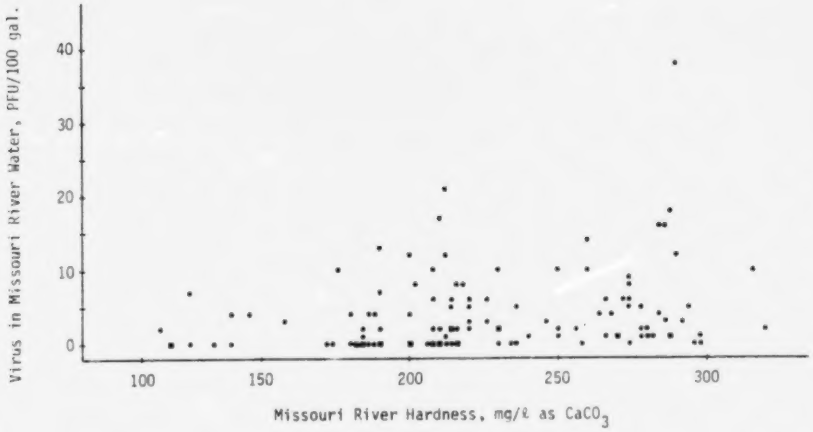

Figure 22 Virus versus Hardness of the Missouri River at Lexington, Missouri

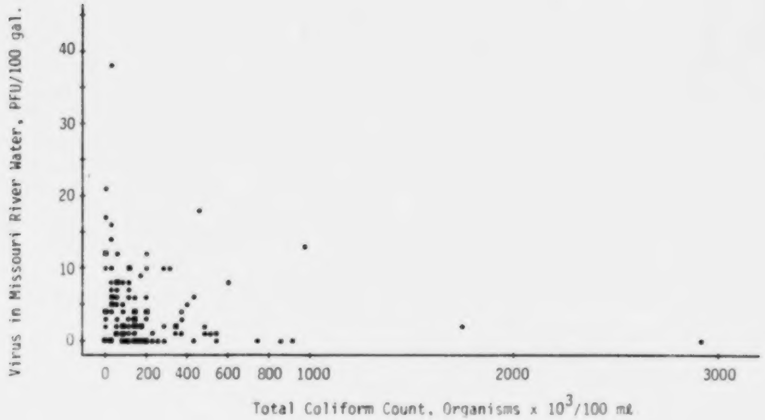

Figure 23 Virus versus Total Coliform Count of the Missouri River at Lexington, Missouri

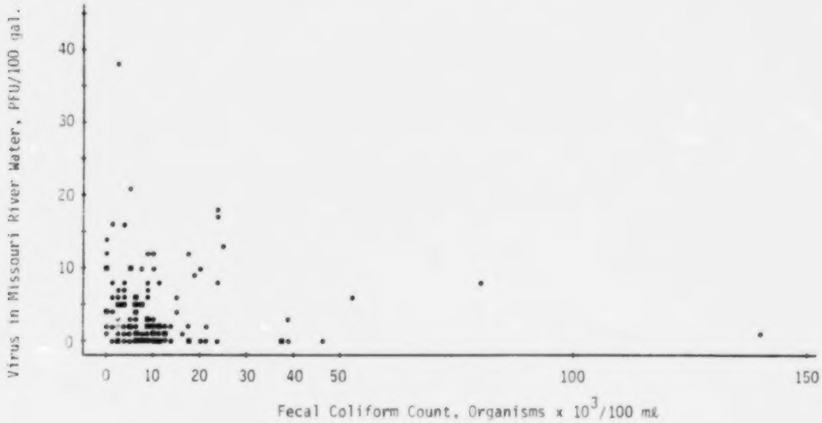

Figure 24 Virus versus Fecal Coliform Count of the Missouri River at Lexington, Missouri

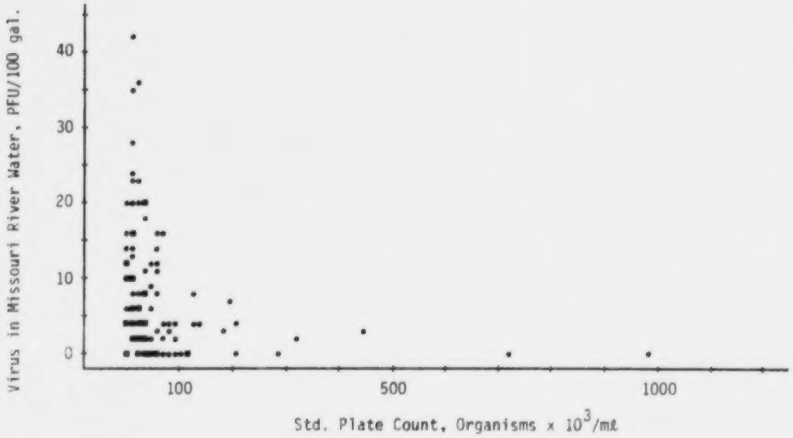

Figure 25 Virus versus Standard Plate Count of the Missouri River at Lexington, Missouri

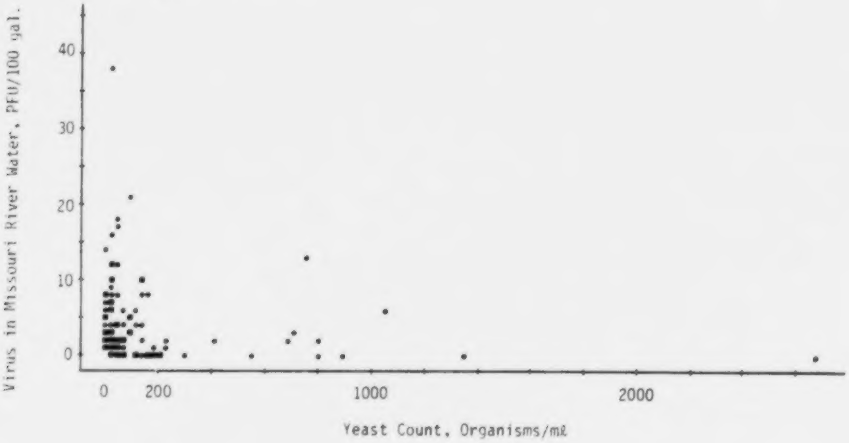

Figure 26 Virus versus Yeast Count of the Missouri River at Lexington, Missouri

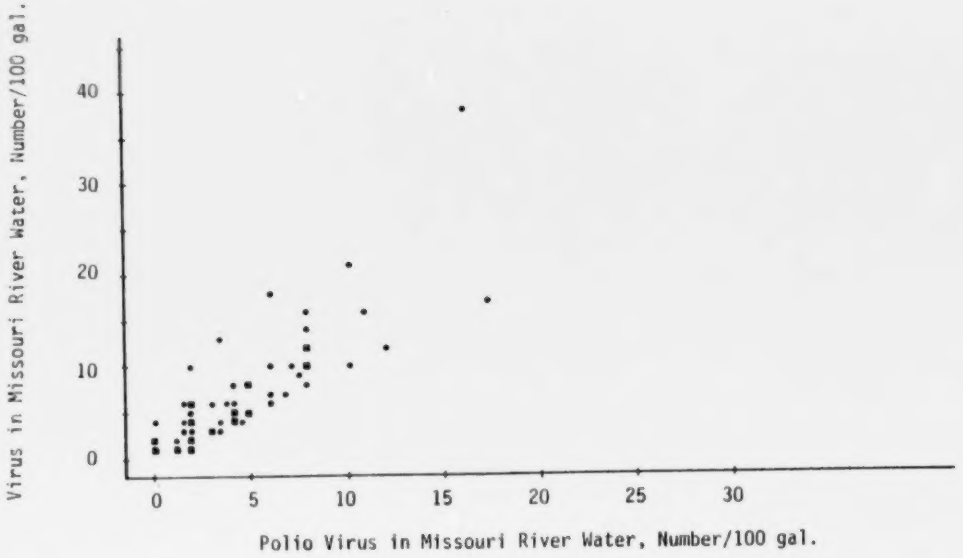

Figure 27 Total Virus versus Poliovirus Count of the Missouri River at Lexington, Missouri

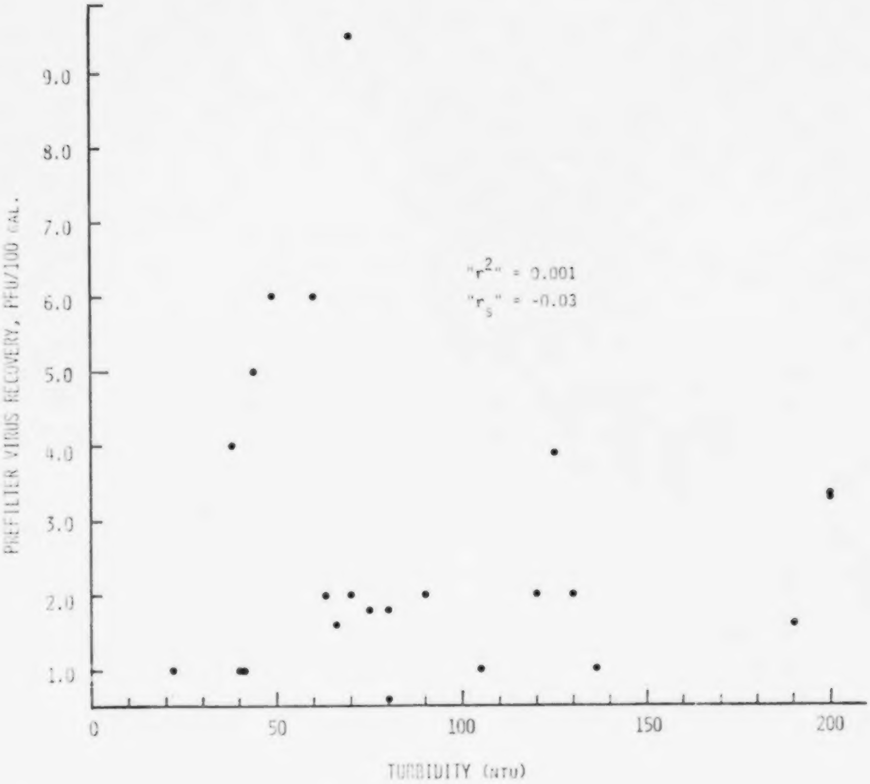

Figure 28 Viruses Recovered from Prefilter versus Turbidity of the Missouri River at Lexington, Missouri

hardness indicate that both are directly related to the virus recoveries as previously observed. Additionally, all "p > F" values ("p > F" being the probability of occurrence by random chance) less than 0.05 indicates that each of the physical, chemical and microbiological correlations are "statistically significant".

Contrary to the Spearman analysis, arithmetic signs for the linear regression coefficients show that all the indicator parameters may be directly correlated with the virus concentrations in the Missouri River. Note, however, that the r^2 values are all below 0.45 and the majority are less than 0.30. These low "r" values indicate that very little of the seasonal variation observed in the virus data can be related linearly to the measured indicator parameters. Moreover, looking specifically at the microbiological parameters, all "p" values (with the exception of the standard plate count correlation) are greater than 0.05 and, therefore, statistically insignificant, i.e., much of the observed linear correlation is probably the result of random chance occurrences.

Neglecting the arithmetic signs, further inspection of the Spearman coefficients shows that, as a group, the physical river parameters are the most highly correlated to the virus concentration data. Chemical parameters are a close second and the microbiological parameters show a large variation in their degrees of correlation.

The linear and Spearman coefficients in Table 4 show some notable correlations among the river water parameters themselves. Again neglecting the arithmetic signs, both the Spearman and linear coefficients indicate that turbidity, alkalinity, hardness, standard plate count and yeast organisms are highly correlated to river flow and are statistically significant. High Spearman values are also noted for all the microbiological parameters when correlated with river temperature and turbidity. Linear coefficients for these correlations, however, only show comparatively large values for the standard plate count and yeast organisms.

The data of Table 4 indicate that the signs of the two statistical analyses are opposing for the alkalinity-to-river flow and hardness-to-river flow correlations. Spearman coefficients indicate an inverse relation (negative sign) while linear coefficients indicate a direct correlation (positive sign).

A summary of all of the virus assay data is provided in Appendix C along with the results of marker tests carried out in vitro on poliovirus isolates to determine virulence. "R" values signifying virulent (wild) poliovirus were obtained for four samples. The remaining isolants indicate poliovirus of low virulence.

Interpretation of Results

The outstanding feature of the results presented in the previous sections is the indication that peak virus concentrations occur in the winter-spring periods. This seasonal distribution is unique because all studies of virus in domestic sewage to date have shown that peak virus concentrations occur in the summer and fall. In fact, epidemologic data supports the sewage investigations in that more cases of enteric disease typically occur in the summer-fall period.

Since domestic sewage is, at the moment, universally accepted as the major source for watercourse viral contamination, the data presented indicates a seasonal discontinuity between virus concentrations in the Missouri River and those in domestic sewage. This discontinuity, however, is not limited to the Missouri River. Data reported by Goyal, et. al., (4) from a study of coastal canal community waters, showed peak virus recoveries occurred in the October-November and March-April months. Other investigators working with natural systems have reported occasional high winter-spring recoveries. In all cases, including the most recent study by Goyal (4), virus samples were only taken on a sporadic basis (one virus sample per week at most) during the investigation. Based on the variability of the raw water virus recoveries as shown in Figure 9, even a bi-weekly virus sampling program may result in an incomplete representation of the seasonal virus levels. Even though the Lexington, Missouri investigation may represent a more detailed picture of seasonal virus levels, it is important to isolate the factors which resulted in the apparent seasonal discontinuity between the Missouri River and urban sewage virus distributions.

One major factor effecting the relation between the Missouri River and the domestic sewage virus data is the flow scheme of each system. Year round discharges of domestic waste water are relatively constant, whereas seasonal flows in the Missouri River are subject to large variations. The summer flow in the Missouri River averages five times that of the winter flow due to seasonal releases of stored water for river navigation needs. Spearman statistical analysis (Table 3) indicates that a significant inverse relation exists between virus concentration and river flow.

The effect of river flow on seasonal virus levels as indicated in the Spearman analysis, is exemplified in the virus data figures normalized for the Missouri River flow variations. Even the normalized data indicate, however, that winter and spring periods still predominate in virus recovery magnitude. Apparently other factors in addition to flow are responsible for the observed seasonal variations.

Many hydraulic characteristics relating to river flow are likely to effect the virus concentration over a short period. For example; Grew, et. al., (5) and Rosen, et. al., (6) have shown that animal viruses antigenically identical to human enteric viruses can be recovered from family pets and food source animals such as cattle, pigs, and sheep. Such findings would indicate that storm water surface runoff could have a pronounced effect on the enteric virus populations in surface waters, such as the Missouri River. An investigation by Berg, et. al., (7) determined that stockyard run-offs did, in fact, yield hundreds of enteric animal viruses antigenically similar to those of human origin. Due to the many different land uses within the Missouri River basin, the effects of surface run-off on the Missouri River virus populations would be largely dependent on the intensity, duration and upstream location of the rainfall.

Aside from hydrologic conditions, many other factors undoubtedly have significant effects on the seasonal virus populations in the Missouri River. For example, O'Brien and Newman (8) concluded from a study of enterovirus inactivation in Rio Grande River water, that temperature was the predominant factor in virus inactivation either directly or in conjunction with heat-activated compounds. The Rio Grande River water used by O'Brien and Newman is almost identical to the Missouri River (at Lexington, Missouri) in its physical, chemical and microbiological characteristics. Based on the conclusions of O'Brien and Newman, the increased water temperatures in the Missouri River during the summer period could account for the lowered virus concentrations.

In addition to temperature-related inactivation, variations in seasonal wastewater treatment efficiency may also affect the river water virus populations. Assuming that

TABLE 3. VIRUS CORRELATION PARAMETERS

Correlation Plot	r_s (+1.0 to -1.0)	$P > \|r_s\|$ (0 to +1.0)	r^2	$p > F$ (0 to +1.0)
V vs Flow	-0.336	0.0001	0.044	0.0059
V vs Temp	-0.328	0.0001	0.089	0.0001
V vs Turb	-0.358	0.0001	0.107	0.0001
V vs Alk	+0.321	0.0004	0.079	0.0010
V vs Hard	+0.283	0.0019	0.049	0.0105
V vs TC	-0.294	0.0005	0.018	0.1236
V vs FC	-0.196	0.0227	0.003	0.5046
V vs SPC	-0.322	0.0003	0.042	0.0240
V vs Y	-0.186	0.0383	0.012	0.1868
V vs PV	+0.889	0.0001	0.285	0.0001

Symbol Key:

V = total virus concentration — number/100 gal.
PV = poliovirus concentration — number/100 gal.

*Flow = river flow — CFS x 10^4
*Temp = river temperature — °C
*Turb = river turbidity — NTU
*Alk = river alkalinity — mg/l as $CaCO_3$ eq
*Hard = river hardness — mg/l as $CaCO_3$ eq
*TC = rtotal coliform count — organisms/ml x 10
*FC = fecal coliform count — organisms/ml x 10
*SPC = standard plate count — organisms/ml x 10^3
*Y = yeast count — organisms/ml x 10

*all samples collected from the Missouri River at Lexington, Mo. corresponding to virus sampling dates.

r_s = Spearman correlation coefficient

$p > |r_s|$ = probability of correlation by random chance

r^2 = simple Linear Regression sample coefficient of determination

(r = linear regression sample correlation coefficient)

P > F = Probability of event occurrence of random chance.

TABLE 4. MIRROURI RIVER FLOW CORRELATION PARAMETERS

Correlation Plot	r_s (+1.0 to -1.0)	$P > \lvert r_s \rvert$ (0 to +1.0)	r^2	p > F (0 to +1.0)
Turb vs Flow	+0.727	0.0001	0.303	0.0001
Alk vs Flow	-0.791	0.0001	0.394	0.0001
Hard vs Hard	-0.754	0.0001	0.388	0.0001
TC vs Flow	+0.370	0.0001	0.011	0.2323
FC vs Flow	+0.324	0.0001	0.035	0.0292
SPC vs Flow	+0.689	0.0001	0.173	0.0001
Y vs Flow	+0.619	0.0001	0.183	0.0001

sporadically high concentrations, had a direct relation to the current health status of the immediate population. From Melnick's hypothesis, the large increase in Coxsackie virus recovery could be attributed to the "water sport situation" if this initial infection became a widespread secondary infection throughout the community.

Virus Indicator Correlations

Spearman "r_s" coefficients for the virus-to-microorganism correlations show inverse relationships.

These results differ from those of Goyal, et. al., (4) and Rai, et. al., (3). Goyal's conclusions state:

> "A strong positive correlation was found between virus concentrations in the water and the MPN of presumptive total coliforms in sediments. No correlation was found between virus concentration and a number of physico-chemical characteristics of water, except, possibly pH."

Alternately, the "r_s" and "r^2" statistical results are indirectly supported by Berg's (14) investigations which conclude that enteroviruses have no precise indicator except for themselves.

Overall, the most important indications of the virus statistical correlations appear to be, as follows:

(1) Definite and significant interrelations exist between the virus concentrations and the physical, chemical, and microbiological characteristics of the Missouri River at Lexington, Missouri.

(2) No one single physical, chemical or microbiological parameter can be used to adequately preduct virus population in the Missouri River at Lexington, Missouri.

Comments on the Virus Recovery Method

No virus concentration procedure is currently accepted as an approved standard method. The 14th edition of Standard Methods lists (as tentative only) the "Microporous Filter Technique" for the detection of enteric viruses in finished water. Since the publication of the 14th ed., several other procedures have been devised for virus concentration. Standard Methods 15th ed. (not officially published at the time of this writing) contains the aluminum hydroxide absorption-precipitation and the polyethylene glyco hydroextractiondialysis methods in addition to microporous filter technique. The point is that virus concentration and detection techniques are still being developed. Therefore, the virus data presented are by no means an unequivocal representation of the populations and distributions of enteroviruses in the Missouri River.

For the purpose of sampling large volumes of polluted water, the Carborundum system was felt capable of providing the best available technology. Several aspects of the "Aquella" concentrating procedure must be noted since they directly relate to the interpretation of the data.

The 0.45 mm pleated filter (virus absorber) used in the concentration process is probably in itself insufficient to remove enteroviruses from water. Although the filter is "treated" to enhance its virus sorptive capacity, the 0.45 micron filter mesh is 15 times greater than the nominal diameter of the largest enterovirus (18 to 30 mm). Recovery of virus particles is enhanced by the addition of aluminum sulfate to form a virus enmeshing aluminum hydroxide precipitate. Whether the aluminum hydroxide precipitate acts to directly enmesh virus particles or form a filter coat reducing the effective pore size, it is apparent that the formation of hydrated aluminum oxide flocs is essential to the satisfactory performance of the virus concentrating system. It seems reasonable to assume that the same factors which affect the efficiency of coagulation/flocculation with aluminum sulfate also affect the virus recovery efficiency. Since the concentration of aluminum sulfate is constant and the characteristics of the Missouri River are variable, it is likely that there was some variation in the virus recovery efficiency of the concentration system.

If it is assumed that the error due to variations in $Al(OH)_3$ adsorption is negligible, the effects on virus survival due to the pH extremes used for the sorption-elution technique must still be questioned.

Several investigators have shown that pH extremes, below 2.5 or above 10.5 will result in 99% inactivation of the enteric viruses. Inasmuch as the virus concentration process employs sorption at pH = 3.5 and elution of pH = 11.5, inactivation of some viruses may occur. Proponents of the two phase system indicate that in tests with seeded enteroviruses inactivation due to pH extremes are minimal. Moreover, laboratory-cultured viruses may not have the same sorption properties as naturally-occurring enteroviruses.

In an investigation to test the sensitivity of a microporous filter method (equivalent to the "Aquella" process) Hill, et. al., (15) showed that virus recovery efficiencies ranged from 28% to 42% at levels of 1 to 2 PFU per 380 liters of water seeded individually with Coxsackie, ECHO, and adenoviruses. Detection reproducability averaged 66% for the three virus species and 95% for poliovirus at levels of 3 to 7 PFU per 380 liters.

Although the results of the Hill study would indicate the two-stage process has good sensitivity, all tests were performed on 1,900 liter volumes of water. Therefore, the reported recovery efficiencies cannot be directly applied to the Missouri River study since only 190 to 380 liters of water were concentrated. A five-fold reduction in sample volume decreases the sample population size proportionately. Statistically, a reduction in the sample population will produce a decrease in the precision and accuracy of results, or in this case, the detection limit of the concentration process in recovering virus.

The combined effects of concentration methodology, sample size and river characteristics could reduce the recovery efficiency of the Aquella process to 5 to 10% or less for the Missouri River study. If so, virus levels in the Missouri River would be 10 to 20 times greater than numerical results reported in this study.

Conclusions

1) Enteroviruses are present in raw Missouri River water at Lexington, Missouri.

2) Virus concentrations appear to show seasonal variations. Peak virus recoveries occurred in the winter and spring seasons with intermittent low recoveries during the summer and fall.

3) Virus concentrations at Lexington, Missouri are substantially effected by seasonal flow variations in the Missouri River. Increased river flow which produces significant dilution effects in the summer and fall as stored water is released for navigation,results in significant dilution of virus.

4) Statistically significant interrelations exist between virus populations in the Missouri River at Lexington, Missouri and physical, chemical and microbiological river characteristics.

5) No individual physical, chemical or microbiological raw water parameter can be used to adequately predict enterovirus population in the Missouri River at Lexington, Missouri. Experimental results indicate that inverse correlations exist between conventional microbiological parameters and virus count.

6) The actual virus populations in the Missouri River at Lexington, Missouri may be many times higher than the numbers presented due to the inherent recovery inefficiency associated with the virus (microporous filter) concentration and analysis procedures.

VIRUS REMOVAL AT LEXINGTON, MISSOURI

Treatment at the Lexington, Missouri, water treatment plant begins with presedimentation. This practice is commonplace among Missouri water utilities owing to consistently high suspended solids in Missouri River water. Raw water turbidities generally range from 50 to 250 NTU with peak values in the thousands. While its chemical composition is variable, Missouri River water is generally hard (200 mg/l as $CaCO_3$ equivalent; 4.0 meq/l), neutral in pH (7.5) and well buffered (alkalinity, 150 mg/l as $CaCO_3$ equivalent; 3.0 meq/l.)

After treatment with lime, either along with or following presedimentation, the water is coagulated with an iron salt, chlorinated, flocculated, settled and filtered.

To study the effectiveness of the Lexington water treatment plant in removing virus, samples were collected both after presedimentation and following the addition of lime. The lime addition raises the pH to roughly 10 and results in a partial precipitation of the carbonate hardness.

Raw Missouri River water samples were taken from the inlet to the presedimentation basin while "presettled" samples were taken at the outlet of the basin. The data of Table 5 show that 39 viruses were recovered from 21 "presettled" water samples, an average of 1.9 PFU/100 gallons. The addition of lime to the presettled water appeared to reduce the number of virus recovered. Only 7 viruses were isolated from 14 samples of lime-treated water for an average of 0.5 PFU/100 gallons. No virus were recovered from 12 of the 14 samples.

TABLE 5. VIRUS RECOVERED FROM SAMPLES TAKEN AT LEXINGTON, MISSOURI, WATER TREATMENT PLANT

Sampling Date	Pre-Settled Water	Lime-Treated Water	Finished Water*	Sampling Date	Pre-settled Water	Lime-Treated Water	Finished Water*
12-2-76	-	0	-	6-22	-	-	0
12-4	-	-	0	6-29	-	-	0
12-9	0	-	-	7-5	-	-	0
12-10	-	0	-	7-13	-	-	0
12-13	-	-	0	7-20	-	-	0
12-15	0	-	-	7-27	-	-	0
12-16	-	0	-	8-3	-	-	0
12-17	-	-	0	8-9	-	0	-
12-20	1	-	-	8-9	-	0	-
12-21	-	0	-	8-10	-	-	0
12-22	-	-	0	8-17	0	0	-
1-5-77	0	-	-	8-17	-	0	-
1-6	-	0	-	8-18	-	-	0
1-8	-	-	0	8-22	1		
1-11	-	-	0	8-23	-	0	-
1-12	-	5	-	8-23	-	0	-
1-13	8	-	-	8-24	-	-	0
1-19	-	2	-	9-8	-	-	0
1-20	-	2	-	9-8	-	-	0
1-21	-	-	0	9-9	0	-	-
2-9	-	-	0	9-12	-	-	0
2-16	-	-	0	9-16**	-	-	0
2-24	-	-	0	9-17	-	-	0
3-2	-	-	0	9-18	-	-	0
3-9	-	-	0	9-20	-	-	0
3-16	-	-	0	9-22	-	-	0
3-23	-	-	0	9-28	-	-	0
3-30	-	-	0	9-24	-	-	0
4-6	-	0	0	9-28	-	-	0
4-15	-	-	0	10-5	-	-	0
4-20	-	-	0	10-12	-	-	0
4-27	-	-	0	10-19	-	-	0
5-4	-	-	0	10-20	1	-	-
5-11	-	-	0	10-21	0	-	-
5-18	-	-	0	10-24	-	-	0
6-3	-	-	0	10-25	0	-	-
6-8	-	-	0	10-26	1	-	-
6-15	-	-	0	10-26	0	-	-

*Of the 71 finished water samples, 65 were analyzed in the laboratories of the U.S. EPA Health Effects Laboratories. The remainder were analyzed by the Carborundum Corporation.

**Onset of period of high flows in the Missouri River due to record (500-1000 year) fainfall in Kansas City area. In ensuing floods, the contents of wastewater treatment plants were lost to Missouri River upstream of Lexington, Missouri.

(continued)

TABLE 5 (continued)

Sampling Date	Pre-settled Water	Lime-Treated Water	Finished Water
10-27	0	-	-
11-9	-	-	0
11-6	-	-	0
11-23	-	-	0
11-30	-	-	0
12-7	-	-	0
12-14	-	-	0
12-21	-	-	0
12-28	-	-	0
1-4-78	-	-	0
1-12	-	-	0
1-18	-	-	0
1-26	-	-	0
2-1	-	-	0
2-8	-	-	0
2-15	-	-	0
2-21	-	-	0
3-1	-	-	0
3-7	0	-	-
3-8	-	-	0
3-9	6	-	-
3-10	0	-	-
3-13	5	-	-
3-14	0	-	-
3-15	-	-	0
3-20	-	-	0
3-22	10	-	-
Total Samples	21	14	71
Total Sample Positive	9	2	0
Total Virus Recovered	39	7	0

No virus were recovered in 71 samples (1.9 m^3) of finished drinking water collected over a 16 month period. Although the results were entirely negative, the sampling data are recorded in Table 5. Of the 71 finished water samples, 65 were sent to Cincinnati, Ohio for assay in the Health Effects Research Laboratories of the U.S. EPA. The remainder were analyzed by the Carborundum Corporation. EPA assayed the samples by inoculating the entire volume onto monolayers of four cell types: BGM, RD, MK and HEK. Response was observed over a 7-day period with one blind passage for a second 7-day period of observation for CPE. The assay procedure employed by the Carborundum Company is presented in Appendix A.

Sampling of the finished water was intensified during the period of September 16-22, 1977, following rainstorms in the Kansas City region which caused record floods and the loss of the contents of regional wastewater treatment plants. Not only did the Lexington finished water exhibit no virus during this period, but virus were undetectable in the raw Missouri River water as well.

The finished water quality at Lexington was very constant over the study period with a final pH between 9 and 10, an alkalinity of 100 mg/l as $CaCO_3$ equivalent (2.0 meq/l), a final hardness of 150 mg/l as $CaCO_3$ equivalent (2.0 meq/l), and an effluent turbidity of less than 0.25 FTU. Fecal and total coliform, yeast and acid-fast bacteria were generally below detection limits, while standard plate counts were less than 20 per ml. Although the Lexington water treatment plant is almost a century old and continues to utilize basins and piping which are antiquated, the plant appeared to be capable of consistently producing good quality water as judged by currently accepted physical, chemical and microbiological standards.

CHEMICAL DISINFECTION FOR VIRUS INACTIVATION

Interspersed with the sampling program for the longitudinal study of virus in Missouri River water, a study was conducted of virus inactivation by various water disinfectants. Because a large sample volume was required for virus concentration, batch disinfection studies were conducted using a 1.9 m^3 polyethylene tank.

Raw Missouri River water, drawn from the presedimentation basin into the polyethylene tank was, first, sampled for virus, and, then, treated with disinfectant. After disinfectant contact periods ranging from 1 to 16 hours, postdisinfection samples were collected. Along with each virus analysis, samples were collected for microbiological analysis, including fecal and total coliform, standard plate count, yeast and acid-fast bacteria.

Disinfectants

Chlorine was applied as sodium hypochlorite. In the chloramine studies, ammonium sulfate, the source of ammonium ion, was applied prior to the addition of sodium hypochlorite.

"Anthium dioxide", a proprietary preparation purported to be a "stabilized, neutral solution of chlorine dioxide," was used in several studies. The effectiveness of this proprietary formulation was compared with that of chlorine dioxide which was freshly prepared in the laboratory using sodium chlorite and chlorine gas.

Ozone was generated on-site using a laboratory-type ozone generator and compressed air. Diffusion of the ozonated air into the polyethylene tank did not result in a measurable ozone residual because of adsorption inefficiencies and the high oxidative demand of the raw Missouri River water.

The results of the virus and microbiological analysis are presented in Table 6.

Chlorine Disinfection

Twenty-one separate studies were conducted using chlorine as a disinfectant. In total, 27 virus were recovered from raw water samples and 5 virus were recovered from the chlorine-treated water. Four virus were recovered when the chlorine dosage was 1 mg/l as chlorine while one virus was recovered when the chlorine dosage was 2 mg/l. In virtually every instance fecal coliform was reduced to below detectable limits, confirming the susceptibility of the fecal coliform organisms to even low dosages of chlorine. In these instances, chlorine was apparently unable to inactivate all of the virus present despite achieving significant destruction of the indicator organisms.

Total coliform organisms were somewhat more resistant to chlorine than the fecal coliform group. However, chlorine dosages in excess of 1 mg/l as chlorine appeared to be capable of reducing total coliform counts to below detectable limits.

Standard plate count organisms were abundant and showed the greatest resistance to disinfection by chlorine. Reductions in standard plate count organisms generally increased to more than two orders of magnitude when chlorine dosages were greater than 1 mg/l as chlorine.

Yeast organisms appeared to be as sensitive to chlorine as the fecal coliform organisms. The number of acid-fast bacteria observed were too small to be useful as an indicator of microbial contamination.

Overall, it appeared that the application of chlorine at dosages up to 2 mg/l resulted in only marginal inactivation of virus. Despite the exceptionally adverse conditions for effective disinfection, most of the conventional indicator organisms in raw Missouri River water were killed by chlorination.

Chloramine Disinfection

Virus appeared to survive chloramine residuals up to 1.6 mg/l as chlorine. However, at chloramine dosages greater than 2.0 mg/l and over prolonged contact periods, virus inactivations were achieved. Again, fecal coliform organisms proved to be most vulnerable to chloramination, being reduced to below detection limits in 13 out of 15 tests. Fecal coliform organisms were completely destroyed even where virus survived.

Total coliform organisms, which were generally an order of magnitude more abundant than fecal coliform in the raw water, were reduced to below detection limits in 8 out of 17 tests. The results were inconsistent, however, with total coliform survivals indicated at high chloramine residuals. Moreover, total coliform survival did not correspond to virus survival. In one case, a virus was isolated from chlorine disinfected water showing an acceptable coliform level. In three other experiments , in which virus were found in disinfected water, high coliform levels also were found.

Standard plate count organisms destruction by chloramination was similarly erratic and did not correspond to virus inactivation. Only one to two order of magnitude reductions in standard plate count organisms were achieved by chloramination. This was far less than that obtained using chlorine alone.

Once more, yeast organisms were about as susceptible as fecal coliform to chloramine disinfection while acid-fast bacteria were often not found in significant numbers.

The disinfection obtained with chloramine in this sequence of 17 batch studies was clearly not as consistent as that obtained in the earlier studies using chlorine. Both the numbers of organisms and their survival may have been influenced by seasonal differences in temperature and water quality.

The chloramine disinfection study of 1/13/78 is of special interest in that virus survival was accompanied by the incomplete destruction of fecal and total coliform as well as only a modest reduction in standard plate count.

Chlorine Dioxide Disinfection

The addition of "Anthium Dioxide", the "stabilized, neutral solution of chlorine dioxide" accomplished little, if any, disinfection. Instead, this series of tests served as a check or control for the analysis of virus and microbiological parameters. Rarely did analysis of raw or treated water samples show differences of as much as one order of magnitude even for sensitive species. More often, the results reproduced within a factor of two despite sampling error and natural die-away. The results indicate that "Anthium Dioxide" does not have water-disinfecting properties.

On the other hand, chlorine dioxide, produced by the reaction between sodium chlorite and chlorine, exhibited strong disinfecting properties when residuals in excess of 1 mg/l as chlorine were established. More definitely than in the studies with chlorine and chloramine, the sequence of destruction of fecal coliform, total coliform and standard plate count organisms was established with chlorine dioxide.

Enteroviruses were isolated in raw Missouri River water in just 4 tests in which chlorine dioxide was used for disinfection. In each instance, no isolates were found in the chlorine dioxide-treated water. Overall, the virus inactivation effectiveness of chlorine dioxide appears to be superior to that of chlorine.

Ozone Disinfection

The dosages of ozone applied for disinfection were exceptionally low, ranging from 0.01 to 0.16 mg/l. The contact period ranged from 5 to 60 minutes, representing the period that air was passed through the ozonator and ozonated air diffused into the test water. Ozone residuals were never detected in the treated water owing to the highly inefficient contact provided in the shallow tank employed in the batch studies.

Despite the unfavorable conditions for disinfection, significant reductions in coliforms and standard plate counts were achieved by dosages as low as 0.08 mg/l ozone applied over a 30 minute period. While viruses in raw Missouri River water were not completely inactivated at low ozone dosages, they were reduced to below the limit of detection at dosages of 0.08 mg/l and higher.

The data on virus and the other microbiological indicators appear to confirm each other in indicating the adequacy or inadequacy of the disinfection treatment. While the presence of coliform groups or large numbers of standard plate count organisms may not indicate the presence of virus, treatment which results in a significant (2 to 3 order of magnitude) reduction in the numbers of these indicator organisms appears to indicate the inactivation of the virus present.

The batch studies of the conventional water disinfectants were limited due to economic and time constraints. However, they seem to confirm the efficacy of conventional water disinfection practices. Despite high raw water turbidities, the lack of pretreatment and variations in disinfectant demand, chlorine dioxide and ozone appeared to be capable of inactivating naturally-occurring human enterovirus. Chlorine and chloramine appeared marginally effective in inactivating virus at lower dosages (2 mg/l). All of the disinfectants, when applied at conventional dosages for conventional contact periods, destroyed most of the microbial indicators of pollution. Considering the comparative susceptability of the coliform organisms, low standard plate counts may have been the best indication of adequate disinfection.

TABLE 6. DISINFECTION OF RAW MISSOURI RIVER WATER

Date	Disin-fectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts					
					Fecal Coliform, No./ 100 ml.	Total Coliform, No./ 100 ml.	+SPC, No./ml	Yeasts, No.l	Acid-Fast No./	Virus Count, PFU/ 100 gal.
1/27/78	*	-	-	-	8,400	125,000	4,120	7	-	3
	Cl_2	0.5	2	0.22	10	16,000	260	2	-	0
1/27/78	*	-	-	-	11,600	167,000	27,000	7	87	0
	Cl_2	0.75	2	-	0	875	138	0	-	0
2/3/78	*	-	-	-	6,200	152,000	8,500	0	-	3
	Cl_2	1.0	2	0.44	0	40	3,120	0	-	2
2/24/78	*	-	-	-	3,000	36,000	2,130	0	-	5
	Cl_2	1.0	2	0.63	0	10	200	0	-	2
3/22/77	*	-	-	-	2,100	17,000	24,000	16	2	0
	Cl_2	1.0	16	-	0	700	3,300	0	2	-
1/20/78	*	-	-	-	6,850	172,000	36,800	36	-	0
	Cl_2	2.0	2	1.33	0	0	106	0	-	0
2/1/78	*	-	-	-	3,800	38,500	4,350	7	127	5
	Cl_2	2.0	2	120	0	0	0	0	-	1
1/18/77	*	-	-	-	1,900	17,000	-	15	-	0
	Cl_2	2.0	4	-	0	500	-	0	-	-
2/23/77	*	-	-	-	85	21,000	6,000	135	120	0
	Cl_2	2.0	6	-	0	0	50	0	75	-

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*Raw Missouri River Water

+Standard Plate Count.

TABLE 6 (continued)

Date	Disin-fectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts					Virus Count, PFU/ 100 gal.
					Fecal Coliform, No./ 100 ml.	Total Coliform, No./ 100 ml.	SPC No./ml	Yeasts, No./ 100 ml	Acid-Fast No./ 100 ml	
5/13/77	*	-	-	-	23,500	578,000	15,000	111	-	0
	Cl_2	2.0	16	0.40	100	46,000	3,100	0	-	0
9/7/77	*	-	-	-	25,000	107,000	260,000	1,180	-	0
	Cl_2	5.0	2	1.18	0	0	9,630	0	-	0
2/8/77	*	-	-	-	1,100	24,000	5,000	30	-	0
	Cl_2	5.0	4	3.78	0	0	80	0	9	-
3/8/77	*	-	-	-	6,700	34,000	5,000	60	0	0
	Cl_2	5.0	8	-	0	0	50	0	0	-
2/15/77	*	-	-	-	5,000	100,000	8,000	45	-	0
	Cl_2	10.0	4	-	0	0	20	0	0	-
3/11/77	*	-	-	-	7,900	25,000	5,400	80	-	5
	Cl_2	10.0	8	-	0	0	6	0	-	-
2/18/77	*	-	-	-	6,000	33,000	17,000	100	-	5
	Cl_2	10.0	16	-	0	0	17	0	7	-
3/4/77	*	-	-	-	11,700	90,000	15,600	150	-	-
	Cl_2	10.0	16	-	0	0	2	0	-	1
3/15/77	*	-	-	-	4,400	8,500	72,000	100	15	-
	Cl_2	15.0	4	-	0	0	370	25	0	1
4/22/77	*	-	-	-	80,000	590,000		130	2	8
	Cl_2	15.0	4	0.75	0	180		0	-	0

*Raw Missouri River water

TABLE 6 (continued)

Date	Disin-fectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts					
					Fecal Coliform, No./100 ml.	Total Coliform, No./100 ml.	SPC No./ml	Yeasts, No./100 ml	Acid-Fast No./100 ml	Virus Count, PFU/100 gal.
5/17/77	*	-	-	-	9,000	405	19,000	18	11	2.9
	Cl_2	15	4	10.66	0	1,900	70	0	0	0
5/20/77	*	-	-	-	210	1,750,000	38,500	22	-	0
	Cl_2	15	16	8.10	0	4,430	253	2	-	-
2/8/78	*	-	-	-	2,300	84,000	17,600	0	0	1
	$Cl_2+NH_4^+$	0.5	2	0.27	0	0	4,300	2	-	0
2/10/78	*	-	-	-	4,000	77,000	12,800	2	-	1
	$Cl_2+NH_4^+$	0.75	2	0.35	-	1,750	1,900	0	-	5
1/13/78	*	-	-	-	3,400	28,000	6,800	17	-	14
	$Cl_2+NH_4^+$	1.0	1	0.72	10	4,400	2,100	4	-	13
2/17/78	*	-	-	-	12,800	90,000	6,700	3	-	2
	$Cl_2+NH_4^+$	1.0	2	0.47	0	15	290	0	-	6
1/10/78	*	-	-	-	9,800	340,000	24,200	20	140	4
	$Cl_2+NH_4^+$	2.0	2	1.60		0	490	0	-	1
2/23/78	*	-	-	-	3,000	36,000	2,200	0	-	8
	$Cl_2+NH_4^+$	2.0	2	1.52	1,600	2,000	66	0	-	0
3/25/77	*	-	-	-	8,800	5,600	24,000	40	-	0
	$Cl_2+NH_4^+$	2.0	4	-	0	0	1,500	2	-	-
5/10/77	*	-	-	-	210	750,000	-	75	4	0
	$Cl_2+NH_4^+$	2.0	4	1.24	0	179,000	-	0	0	0

*Raw Missouri River water

TABLE 6 (continued)

Date	Disinfectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts					
					Fecal Coliform, No./ 100 ml.	Total Coliform, No./ 100 ml.	SPC No./ml	Yeasts, No./ 100 ml	Acid-Fast No./ 100 ml	Virus Count, PFU/ 100 gal.
4/26/77	*	-	-	-	7,300	400,000	9,200	0	28	0
	$Cl_2+NH_4^+$	2.0	8	1.49	0	0	680	0	0	-
3/29/77	*	-	-	-	7,700	3,200	15,600	36	5	0
	$Cl_2+NH_4^+$	5.0	4	-	0	0	510	0	1	-
4/29/77	*	-	-	-	12,000	70,000	16,000	8	-	1
	$Cl_2+NH_4^+$	5.0	8	3.77	0	12,800	-	0	-	-
4/2/77	*	-	-	-	11,000	920,000	-	13	-	0
	$Cl_2+NH_4^+$	5.0	16	-	0	0	360	0	-	-
4/5/77	*	-	-	-	8,900	160,000	3,500	15	-	0
	$Cl_2+NH_4^+$	10.0	4	-	0	0	370	0	-	-
5/3/77	*	-	-	-	2,400	54,000	17,500	23	21	3
	$Cl_2+NH_4^+$	10.0	8	4.35	0	17,000	-	0	2	0
4/14/77	*	-	-	-	3,000	11,400	2,100	5	3	0
	$Cl_2+NH_4^+$	10.0	16	-	0	20	90	0	2	-
5/6/77	*	-	-	-	10,500	2,910,000	21,300	150	-	0
	$Cl_2+NH_4^+$	15.0	8	2.38	0	0	1,400	0	-	0
4/19/77	*	-	-	-	8,500	21,000	5,000	9	23	0
	$Cl_2+NH_4^+$	15.0	16	0.20	0	1,000	1,500	0	4	-
6/2/77	*	-	-	-	37,000	865,000	112,000	217	1130	0
	Anth-O_2	1.0	4	0.71	6,400	246,000	115,000	56	-	0

*Raw Missouri River water

TABLE 6 (continued)

Date	Disin-fectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts					Virus Count, PFU/100 gal.
					Fecal Coliform, No./100 ml.	Total Coliform, No./100 ml.	SPC No./ml	Yeasts, No./100 ml	Acid-Fast No./100 ml	
6/21/77	*	-	-	-	20,000	210,000	291,000	150	-	0
	Anth-0_2	1.0	16	0.41	8,100	88,000	155,000	127	-	-
5/24/77	*	-	-	-	13,900	81,300	-	123	0	0
	Anth-0_2	2.0	4	-	38,000	110,000	12,000	106	0	0
5/27/77	*	-	-	-	2,100	158,000	71,500	32	-	0
	Anth-0_2	2.0	8	1.72	173	-	21,000	59	-	-
6/24/77	*	-	-	-	14,400	154,000	121,000	61	-	0
	Anth-0_2	2.0	16	0.84	4,900	319,000	93,000	108	0	-
6/28/77	*	-	-	-	8,400	137,000	114,000	62	250	0
	Anth-0_2	4.0	16	2.70	1,700	22,000	-	42	250	-
6/7/77	*	-	-	-	6,100	98,500	33,000	32	1.400	0
	Anth-0_2	5.0	4	0.71	4,200	25,000	17,100	18	0	-
6/10/77	*	-	-	-	10,000	139,000	44,000	11	-	0
	Anth-0_2	5.0	8	4.37	4,000	195,000	40,000	-	-	0
6/17/77	*	-	-	-	6,500	131,000	43,000	11	-	4.8
	Anth-0_2	10.0	4	9.19	11,000	2,500	56,000	18	-	6.7
7/19/77	*	-	-	-	68,500	11,500	13,200	11	0	1.6
	Anth-0_2	10.0	8	8.28	3,800	23,700	10,300	6	0	1.6
7/22/77	*	-	-	-	4,500	77,000	450,000	21	-	1.5
	Anth-0_2	10.0	16	7.97	5,000	70,000	11,300	48	-	0

*Raw Missouri River water

TABLE 6 (continued)

Date	Disinfectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts					Virus Count, PFU/ 100 gal.
					Fecal Coliform, No./ 100 ml.	Total Coliform, No./ 100 ml.	SPC No./ml	Yeasts, No./ 100 ml	Acid-Fast No./ 100 ml	
7/26/77	*	–	–	–	6,500	64,000	61,000	29	20	1.2
	Anth-0$_2$	15.0	8	12.49	2.100	127,000	7,700	18	–	0
7/29/77	*	–	–	–	5,400	84,000	19,000	7	–	2.2
	Anth-0$_2$	15.0	16	12.32	4,300	185,000	16,500	17	–	0
10/11/77	*	–	–	–	12,500	154,000	58,000	3000	550	0
	C10$_2$	0.5	4	0.20	2,800	29,000	107,000	870	–	0
9/20/77	*	–	–	–	4,600	82,000	106,000	42	–	2
	C10$_2$	0.5	16	–	890	23,500	70,000	26	–	0
9/27/77	*	–	–	–	10,000	91,300	120,000	900	60	0
	C10$_2$	1.0	4	0.33	3,900	32,000	–	615	–	0
10/14/77	*	–	–	–	8,700	190,000	91,000	208	–	0
	C10$_2$	1.0	4	0.71	0	1,600	1,700	0	–	0
9/22/77	*	–	–	–	17,000	130,000	980,000	143	–	0
	C10$_2$	1.0	16	0.63	110	4,200	162,000	12	–	–
10/18/77	*	–	–	–	39,000	155,000	14,700	41	487	0
	C10$_2$	1.5	4	0.81	0	75	500	0	–	0
9/30/77	*	–	–	–	13,300	170,000	122,000	240	–	2
	C10$_2$	2.0	4	1.25	0	292	3,700	0	–	0
10/21/77	*	–	–	–	8,400	495,000	74,000	40	–	–
	C10$_2$	3.0	4	2.55	0	0	26	0	–	–

*Raw Missouri River water

TABLE 6 (continued)

Date	Disin-fectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts Fecal Coliform, No./ 100 ml.	Total Coliform, No./ 100 ml.	SPC, No./ml	Yeasts, No.l 100 ml	Acid-Fast No./ 100 ml	Virus Count, PFU / 100 gal.
10/7/77	*	-	-	-	9,000	225,000	15,000	128	-	2
	ClO_2	4.0	4	3.51	0	0	32	0	-	0
10/3/77	*	-	-	-	2,500	90,500	6,100	28	0	2
	ClO_2	5.0	4	2.73	0	0	17	0	-	0
11/11/77	*	-	-	-	25,000	970,000	-	755	-	10
	O_3	0.01**	5 min	NM	15,500	485,000	4,700	975	-	4
11/15/77	*	-	-	-	53,000	204,000	60,000	1040	100	4
	O_3	0.04	15 min	NM	27,000	130,000	71,000	660	-	0
12/15/77	*	-	-	-	11,800	280,000	17,500	40	-	0
	O_3	0.04	15 min	NM	10	1,140	580	0	-	-
11/22/77	*	-	-	-	9,800	200,000	14,600	84	120	3
	O_3	0.05	20 min	NM	153,000	3,400	40,000	46	-	2
12/20/77	*	-	-	-	8,300	153,000	16,200	27	105	0
	O_3	0.05	20 min	NM	0	165	87	0	-	-
11/18/77	*	-	-	-	14,700	415,000	36,000	119	-	2.9
	O_3	0.07	25 min	NM	8,800	169,000	23,000	150	-	2.1
12/23/77	*	-	-	-	16,500	375,000	30,600	225	-	1
	O_3	0.07	25 min	NM	0	75	205	0	-	-

*Raw Missouri River water

**Ozone dosage is the total amount applied, not adsorbed

NM-Not Measurable

TABLE 6 (continued)

Date	Disin-fectant	Dosage, mg/l	Contact Time hrs.	Residual, mg/l	Bacterial Counts Fecal Coliform, No./ 100 ml.	Total Coliform, No./ 100 ml.	SPC, No./ml	Yeasts, No.l 100 ml	Acid-Fast No./ 100 ml	Virus Count, PFU/ 100 gal.
11/30/77	*	-	-	-	6,600	132,000	19,500	47	-	4.1
	O_3	0.08**	30 min	NM	60	3,600	210	6	-	0
12/9/77	*	-	-	-	-	360,000	41,000	35	-	4.4
	O_3	0.08	30 min	NM	0	207	138	0	-	0
12/13/77	*	-	-	-	9,900	192,000	17,500	29	60	0
	O_3	0.12	45 min	NM	0	125	24	0	-	-
12/2/77	*	-	-	-	18,500	173,000	33,000	30	-	8.8
	O_3	0.16	60 min	NM	150	1,100	700	0	-	0

*Raw Missouri River water

**Ozone dosage is the total amount applied, not absorbed

NM-Not Measurable

SECTION 6

MOBILE PILOT PLANT STUDIES

INTRODUCTION

The fourth major task of the overall project required the design, construction and operation of dual pilot-scale, mobile water treatment plants. The objective was to develop a facility which could be easily transported to conduct research on the treatability of various waters at different locations. Treatability of a specific water would be determined by monitoring the quality of the pilot plant effluents under different operating conditions.

PILOT PLANT DESIGN AND CONSTRUCTION

Several prerequisite functional characteristics served as guidelines for the design of the pilot water treatment facility:

1) The entire system was to be self-contained and readily transportable.

2) The system was to model conventional physicochemical processes used by most U.S. drinking water treatment facilities.

3) The system was to operate on a continuous-flow basis with sufficient flexibility to allow simultaneous comparison of individual unit processes or entire water treatment schemes.

4) The system was to provide broad spectrum capabilities for conducting research on various water quality parameters (e.g.; viruses, microorganisms, particulates, trace organic and inorganic substances).

The final design proposed the construction of two identical treatment systems utilizing rapid chemical mixing, coagulation/flocculation, sedimentation, filtration and treated water storage. System outputs were independently variable up to a maximum treatment rate of 27 m^3/day (5 gpm) yielding a total plant capacity of 54.5 m^3/day. Major treatment units were constructed of stainless steel. Integral plumbing, pumping and flow control devices (including all physical contact surfaces) consisted of stainless steel, glass, teflon or tygon materials. All unit processes and related equipment were designed so as to be completely contained within a standard 12.2 m tractor-trailer.

Unit process tanks were fabricated (Helium arc-welded) from 3 mm thick 316-stainless steel sheets. All units were detailed, assembled and tested in the Civil Engineering Laboratory/shop area. Volumes and detention times for each unit process are listed in Table 7. Following testing, approved units were permanently installed within the tractor-trailer.

Water to be processed enters at the rear of the trailer and is pumped via an overhead pipe into a constant head tank. The rapid mixing chambers for each system are gravity fed from the constant head tank. Water flows by gravity from the rapid mix units through the flocculation and sedimentation processes, respectively. Following collection from the sedimentation tank weir, settled water is centrifugally pumped to two filter columns which operate in a pressurized down-flow mode. Filters may be used in either parallel or series operation. Filtered water can be collected in storage tanks for post-treatment or it may be discharged directly into the plant's drainage system. In addition to providing for conventional treatment utilizing sedimentation, each system has by-pass plumbing incorporated for ready conversion to a direct filtration operating mode. The two systems may also be interconnected to provide for series treatment, such as two-stage lime softening.

OPERATION OF PILOT PLANT AT LEXINGTON, MISSOURI

For inaugural field operation and testing, the mobile pilot plant was transported to the Lexington Water Treatment Plant where all previous phases of the virus study were conducted. The objective of the Lexington, Missouri, pilot plant study was to assess the effectiveness of *physical* water treatment processes for the removal of viruses and microorganisms. Specifically, the virus removal capability of *direct filtration* using an organic polymer coagulant was to be compared with *conventional treatment* (i.e., coagulation/flocculation-sedimentation-filtration) using an inorganic metal coagulant. No chemical disinfectants were applied at any point within either treatment system.

The influent to the pilot plant was presettled Missouri River water from the Lexington plant. Presedimentation of the influent water was required owing to the large quantities of sand and silt in the raw Missouri River water. The effect of presedimentation on initial virus removal was considered to be minimal based on the results of several researchers (Boardman, et. al., (16), Sherman, et. al. (17), and Clark, et. al. (18) and the indication from the longitudinal study that only 10% of the Missouri River enteroviruses were "solids associated" (Section I, part 3.4). Influent water was continuously treated by each plant system operating at the maximum 5 gpm (27.3 m^3/day) design flow.

Conventional treatment was initiated with aluminum or ferrous sulfate addition to the rapid mix unit. Thereafter, approximately 30 minutes of flocculation and four hours of settling were provided. For a comparison of efficiency, the dual filters were operated in parallel. One filter contained only sand (effective size 0.5 mm) while the other filter contained sand plus anthracite coal (effective size 0.8 mm). Filtration rates were maintained at 13.7 m^3/d (2.5 gpm; 3.5 gpm/sf) for an average of eight hours per day. Backwashing followed turbidity breakthrough. Relative breakthrough was considered to have occurred when turbidity levels exceeded two times the initial value recorded 30 minutes after filter start-up.

Direct filtration was assisted by the addition of a cationic polymer (nominal molecular wt. = 500,000) to the pilot plant's second rapid mix unit. The polymer-treated water was flocculated at moderate "G" levels for approximately 40 minutes. The direct filtration protocol, following flocculation, was identical to that for con-

TABLE 7. HYDRAULIC CHARACTERISTICS OF PILOT PLANT UNIT PROCESSES (VALUES BASED ON 27 m^3/DAY MAXIMUM DESIGN FLOW)

Unit Process	Volume, m^3 (gallons)	Theoretical Detention Time Minutes	*Actual Detention Time, Minutes
Rapid Mix Tank	0.04 (10)	2	1.5
Flocculator	0.76 (200)	40	20**
Clarifier	5.7 (1500)	300	210 - 300***
Filter Column	0.11 (30)	6	6
Clearwell	0.76 (200)	40	10

*Determined with $KMnO_4$ dye study

**Depending on number and speed of the four paddles operating

***Depending on the horizontal and vertical position of the Collection weir.

ventional treatment with the exception that the direct filtration rate was reduced to 5.5 m^3/day (1 gpm; 1.5 gpm/sf).

Virus, Bacteriophage and Microorganism Analyses

Raw Missouri River virus samples collected and analyzed as part of the longitudinal study also served as pilot plant influent samples. Plant effluent samples were similarly collected and processed by the Carborundum Company (Appendix A). In order to perform the analysis for effluent virus concentrations, filtered pilot plant water was collected in the respective clearwells and subsequently pumped to a 1.9 m^3 (500 gallon) holding/sampling tank. Using this procedure, no comparison could be made between the performance of single and dual media filters with respect to virus removal. Recorded data were accordingly designated "filt" to indicate the combined filter output of the clearwell samples.

Analyses for naturally-occurring MS_2 bacteriophage were conducted in conjunction with the raw Missouri River and pilot plant effluent enterovirus samples. University of Missouri-Columbia laboratory staff received one milliliter of the Carborundum final virus concentrate to be used for bacteriophage enumerations. "Bacteriophage" concentrates were serially diluted and plated onto E. Coli Hfr^+ strain bacteria cultures. Plaques were counted six to ten hours after nutrient agar overlay and incubation. All laboratory analyses for MS_2 phage enumerations were in strict accordance with methods contained in "Bacteriophage Techniques", Methods in Virology, Volume 1 (19).

Microbiological analyses consisted of Fecal Coliform, Total Coliform, Standard Plate Count, Yeast and Fecal Streptococci enumerations. Analyses were performed by the UMC Civil Engineering laboratory staff following the procedures outlined in Standard Methods (1). Specific sample collection and shipping techniques (also in accordance with Standard Methods procedures) were identical to those described in Section I, part 2.

LEXINGTON PILOT PLANT RESULTS

Lexington pilot plant studies were conducted between March 1, 1978 and July 31, 1978. Data collected over the five month period can be separated into three specific, yet interrelating portions. The first portion of the study encompassed the month of March and focused on the removal of naturally-occurring enteroviruses and MS_2 bacteriophage. The second portion of the study evaluated only the removal of indicator microorganisms due to anticipated low influent water enterovirus concentrations predicted to begin in April. These microorganism removal studies were continued through the end of May while preparations were being made for the final portion of the Lexington pilot plant study. Since the overall objective was to observe virus particle removals through physical treatment, the third study portion consisted of "seeding" the pilot plant influent water with large numbers of laboratory-cultured MS_2 strain bacteriophage.

Data collected for evaluating the removal of naturally-occurring virus are presented in Table 8. Over the 30-day study period, nine raw water and sixteen pilot plant effluent samples were concentrated and assayed for viruses. Nine out of nine raw Missouri River concentrates exhibited positive enterovirus recoveries while five out of the nine yielded MS_2 phage enumerations. Concurrently, 12 of the 16 effluent concentrates revealed positive enterovirus recoveries and 6 out of 16 samples contained bacteriophage.

Influent microbial indicator organisms ranged from as low as 15 organisms per 100 ml to more than $13x10^6$ microorganisms per 100 ml. Standard Plate Count and Yeast Organisms consistently represented the high and low influent microorganism concentrations, respectively. Total Coliforms and Fecal Streptococci organisms showed similar influent water concentrations each ranging from 15,000 to 200,000 counts per 100 ml. Fecal coliform levels were typically about 10% of the influent total coliform concentrations.

Presettled Missouri River water turbidity ranged between 19 NTU and 110 NTU. When raw river water turbidities exceeded 200 NTU, lime was added to the influent of the Lexington water treatment plant presedimentation basin. The lime addition increased the pH of the pilot plant influent from 7.8 ± 0.2 to 10.3 ± 0.5. This increased pH level appeared to have a minimal effect on the naturally-occurring virus and microorganism concentrations.

MS_2 Bacteriophage and Enterovirus Removals

While MS_2 bacteriophage removals of approximately one order of magnitude were consistently achieved by both conventional treatment and direct filtration as indicated in Table 9, enterovirus removals were low. Only half of the naturally-occurring human enterovirus were removed by conventional treatment despite high removals of suspended solids (turbidity) and indicator organisms. Direct filtration was less effective than conventional treatment, achieving only 17% removal of enterovirus in the first series of tests using presettled Missouri River water without the addition of lime. The addition of lime during periods of high raw water turbidity had little apparent effect on MS_2 bacteriophage and enterovirus removals except for the apparent improvement in the performance of direct filtration with respect to enterovirus removals. Even so, only one-half of the enterovirus were removed.

Turbidity Removals

Table 10 shows the reductions in turbidity achieved by conventional treatment (coagulation, flocculation, sedimentation and filtration) and direct filtration during four series of experiments. Considering turbidity removals only, both processes appeared to be comparable but only marginally effective in meeting the MCL for turbidity. The MCL was achieved only in the fourth series of experiments. Still, prior to the acquisition of the data on virus removals, the turbidity reductions were considered to be an indication of effective virus removal.

Indicator Microorganism Removals

The removals of fecal coliform, total coliform, standard plate count organisms, yeast and fecal streptococcus, as shown in Table 11, were remarkably consistent. Generally, conventional treatment achieved one to two order of magnitude reductions in all indicator organisms. The addition of lime in experimental series 2 resulted in enhanced organism reductions.

The difference between the performance of direct filtration and conventional treatment was clearly evident from the indicator organism removals. Generally, direct filtration was not as effective as conventional treatment, achieving less than an order of magnitude reduction in the number of microorganisms. As in most of the studies, the standard plate count organisms were the most abundant and persistent, making them the most useful group of organisms for observing the comparative effectiveness of competing water treatment processes.

The effective removal of the indicator organisms confirmed the indication provided by turbidity reductions that significant virus reductions were being achieved. The marginal removal of the naturally-occurring enterovirus was not known until well after the results of other analyses, as well as the experiments themselves, were complete.

Removal of Laboratory-Cultured MS_2 Bacteriophage

Laboratory-cultured MS_2 bacteriophage did not survive introduction to the Missouri River water very well. Initial MS_2 seedings at concentrations of 1×10^6 phage per 100 liters of influent water yielded no effluent (or intermediate unit process) bacteriophage recoveries. Kinetic die-away studies (Appendix E) subsequently revealed that all but 4.78×10^6 percent of the seed population, i.e., one phage particle out of every 210,000 applied, survived. High density MS_2 cultures were prepared and influent water phase concentrations were estimated based on the percent survival of the known seed culture population density. Data collected for the high density "seeded" virus studies are shown in Table 12.

Not only were most of the laboratory-cultured virus inactivated as soon as they were added to the river water, but the apparent removals of the seeded organisms were far greater than the comparable removals of the naturally-occurring enterovirus and bacteriophage.

The implication of these results is that seeded organisms may not be well-adapted for survival in natural waters. If so, they may not serve well to evaluate plant performance with respect to the removal of naturally-occurring virus.

While seeded MS_2 bacteriophage removals were two orders of magnitude under normal conditions, the addition of lime resulted in their total destruction. Again, this did not coincide with the results obtained with naturally-occurring virus which showed little response to the addition of lime.

In summary, it appears that the removal of naturally-occurring human enterovirus and MS_2 bacteriophage is not mirrored by the removal of turbidity, conventional indicator organisms or seeded laboratory-cultured MS_2 bacteriophage. Moreover, conventional physical water treatment processes appear to have limited effectiveness in removing naturally-occurring human enterovirus from Missouri River water, with or without the addition of lime.

TABLE 8. RESULTS OF WATER SAMPLE ANALYSES FROM LEXINGTON, MISSOURI PILOT PLANT OPERATION

Date	Sample	pH	Turbidity (NTU)	Coagulant	Dosage (mg/l)	Indicator Organism Counts					Virus Count 100 gal.	MS$_2$ Phage Count PFU/ 100 gal.
						Fecal Coli. #/100 ml	Total Coli. #/100 ml	Std. Plate #/ml	Yeast Ccunt #/100 ml	Fecal Strep. #/100 ml		
3/2/78	Influent	7.8	40	-	-	10,400	193,000	27,000	27	-	11.7	7,175
	Conv. Filt	7.6	-	$Al_2(SO_4)_3$	50	-	-	-	-	-	7	-
3/6/78	Influent	7.9	22	-	-	4,500	128,000	31,500	15	232,000	10	-
3/8/78	Conv. Filt.	-	1.2	$Al_2(SO_4)_3$	50	1,250	18,600	1,100	0	40,500	5	900
	Dir. Filt.	-	2.2	Polymer	2	1,300	20,300	9,000	10	19,700	9	-
3/9/78	Influent	7.8	19	-	-	2,800	63,300	15,200	32	14,600	6.4	400
	Conv. Filt.	7.8	1.4	$Al_2(SO_4)_3$	50	200	4,500	1,400	0	0	0	173
	Dir. Filt.	7.7	1.8	Polymer	2	1,350	10,000	3,300	0	9,400	5.4	483
3/13/78	Influent	7.9	110	-	-	3,600	112,000	56,500	33	98,000	6.8	-
	Conv. Filt.	7.3	2.8	$Al_2(SO_4)_3$	100	400	12,600	19,600	2	5,500	1	0
	Dir. Filt.	7.8	8.5	Polymer	4	1,600	34,500	57,000	6	51,500	-	-
3/15/78	Influent	7.7	105	-	-	4,400	184,000	81,000	76	250,000	1.7	162
	Conv. Filt.	7.1	1.0	$Al_2(SO_4)_3$	100	0	15,100	3,700	0	2,300	2.9	0
	Dir. Filt.	7.7	4.5	Polymer	4	200	9,900	17,400	0	8,900	4	-
3/20/78	Influent + Lime	10.4	105	-	-	100	15,300	35,000	24	53,800	9.3	1,017
	Conv. Filt.	10.2	1.0	$FeSO_4$	80	0	100	2,700	0	2,600	3	0
	Dir. Filt.	10.1	5.6	Polymer	4	10	1,100	23,000	1	8,600	4	200

TABLE 8 (continued)

Date	Sample	pH	Turbidity (NTU)	Coagulant	Dosage (mg/l)	Indicator Organism Counts					Virus Count 100 gal.	MS_2 Phage Count PFU/ 100 gal.
						Fecal Coli. #/100 ml	Total Coli. #/100 ml	Std. Plate #/ml	Yeast Count #/100 ml	Fecal Strep. #/100 ml		
3/21/78	Influent + Lime	10.6	53	-	-	1,350	46,000	134,000	40	134,000	1.4	-
	Conv. Filt.	8.9	0.9	$FeSO_4$	80	0	0	310	0	600	0	0
	Dir. Filt.	10.4	2.9	Polymer	4	0	0	2,000	0	3,700	1.2	190
3/28/78	Influent + Lime	10.1	51	-	-	7,000	82,800	95,000	50	960,000	1.5	-
	Conv. Filt.	10.1	7.1	$FeSO_4$	80	0	0	320	0	490	0	-
	Dir. Filt.	10.2	1.4	Polymer	4	0	0	320	0	1,100	0	-
3/30/78	Influent + Lime	9.9	51	-	-	5,100	150,000	42,000	81	14,300	1.2	1140
	Conv. Filt.	9.4	4.8	$FeSO_4$	90	0	30	1,200	0	240	3	330
	Dir. Filt.	9.9	2.6	Polymer	4	0	0	560	0	300	1	90
4/10/78	Influent	7.5	125	-	0	1,400	33,000	9,200	5	78,000	-	-
	Conv. Filt.	7.1	3.6	$Al_2(SO_4)_3$	65	180	3,800	990	0	5,300	-	-
	Dir. Filt.	7.5	2.7	Polymer	4	310	2,800	1,100	1	3,500	-	-
4/13/78	Influent	7.5	180	-	-	3,900	27,000	20,000	48	68,000	-	-
	Conv. Filt.	7.4	3.8	$Al_2(SO_4)_3$	90	-	1,500	1,200	0	2,600	-	-
	Dir. Filt.	7.4	77	Polymer	4	-	3,700	5,700	0	10,000	-	-
4/18/78	Influent	7.5	105	-	-	2,400	32,000	14,000	16	30,000	-	-
	Conv. Filt.	7.0	1.8	$Al_2(SO_4)_3$	100	100	1,200	620	0	440	-	-
	Dir. Filt.	7.4	0.4	Polymer	4	450	3,300	730	0	1,500		

TABLE 8 (continued)

Date	Sample	pH	Turbidity (NTU)	Coagulant	Dosage (mg/l)	Indicator Organism Count Fecal Coli. #/100 ml	Total Coli. #/100 ml	Std. Plate #/ml	Yeast Count #/100 ml	Fecal Strep. #/100 ml	Virus Count PFU/ 100 gal.	Seeded MS_2 Phage Count, PFU/ 100 gal.
4/20/78	Influent	7.5	200	-	-	12,000	68,000	56,000	142	130,000	-	no seed
	Conv. Filt.	7.4	1.6	$Al_2(SO_4)_3$	100	1,000	4,300	3,100	0	7,700	-	-
	Dir. Filt.	7.4	5.8	Polymer	4	590	2,800	880	0	2,300	-	-
4/25/78	Influent	7.7	130	-	-	2,500	23,000	21,000	22	123,000	-	no seed
	Conv. Filt.	7.6	6.6	$Al_2(SO_4)_3$	100	210	1,800	330	0	710	-	-
	Dir. Filt.	7.6	4.3	Polymer	4	410	9,700	1,400	0	14,000	-	-
4/27/78	Influent	7.7	70	-	-	2,600	70,000	16,300	29	117,000	-	no seed
	Conv. Filt.	7.5	1.0	$Al_2(SO_4)_3$	100	25	210	150	0	400	-	-
	Dir. Filt.	7.7	0.4	Polymer	4	490	4,500	2,000	0	8,400	-	-
5/2/78	Raw River	-	130	-	-	3,600	63,000	48,000	55	144,000	-	no seed
	Influent	8.0	47	-	-	1,900	19,000	22,000	4	150,000	-	-
	Conv. Filt.	7.9	0.9	$Al_2(SO_4)_3$	100	35	160	340	0	560	-	-
	Dir. Filt.	7.9	0.3	Polymer	4	240	1,800	1,100	2	2,000	-	-
5/9/78	Raw River	7.9	-	-	-	14,000	94,000	111,000	126	190,000	-	no seed
	Influent	7.8	120	-	-	1,800	28,000	6,100	7	13,000	-	-
	Conv. Filt.	7.2	0.6	$Al_2(SO_4)_3$	100	350	2,700	1,600	1	2,500	-	-
	Dir. Filt.	7.8	1.8	Polymer	4	940	8,300	11,300	1	15,000	-	-
5/16/78	Raw River	7.8	170	-	-	7,500	81,000	60,000	57	320,000	-	no seed
	Influent	7.7	68	-	-	2,700	12,000	13,000	0	42,000	-	-
	Conv. Filt.	7.2	0.3	$Al_2(SO_4)_3$	100	15	200	1,900	0	200	-	-
	Dir. Filt.	7.7	0.2	Polymer	4	500	1,200	840	0	8,300	-	-

TABLE 8 (continued)

Date	Sample	pH	Turbidity (NTU)	Coagulant	Dosage (mg/l)	Indicator Organism Counts					Virus Count PFU/ 100 gal.	Seeded MS_2 Phage Count, PFU/ 100 gal.
						Fecal Coli. #/100 ml	Total Coli. #/100 ml	Std. Plate #/ml	Yeast Count #/100 ml	Fecal Strep. #/100 ml		
5/23/78	Raw River	7.7	150	-	-	5,000	98,000	42,000	19	118,000	-	no seed
	Influent	7.8	46	-	-	1,100	19,000	6,100	0	24,000	-	-
	Conv. Filt.	6.9	0.2	$Al_2(SO_4)_3$	100	0	100	610	0	190	-	-
	Dir. Filt.	7.8	0.3	Polymer	4	310	1,700	1,800	0	4,700	-	-
5/31/78	Raw River	7.9	180	-	-	5,100	39,000	41,000	48	126,000	-	-
	Influent	8.0	74	-	-	2,000	25,000	14,000	8	33,000	-	no seed
	Conv. Filt.	7.4	0.2	$Al_2(SO_4)_3$	100	20	160	1,000	0	640	-	-
	Dir. Filt.	8.0	0.2	Polymer	4	1,900	17,000	6,700	0	2,700	-	-
7/5/78	Influent	-	31	-	-	1,000	11,000	6,600	1	5,700	-	$2.5x10^7$
	Settled	-	4.7	$Al_2(SO_4)_3$	100	250	3,200	10,000	0	28,000	-	$8.0x10^4$
	Conv. Filt.	-	0.4	"	"	10	200	6,500	0	35	-	$2.0x10^4$
	Dir. Filt.	-	0.2	Polymer	4	50	880	5,200	0	330	-	$9.0x10^4$
7/10/78	Raw River	-	154	-	-	7,400	102,000	142,000	76	59,000	-	no seed
	Influent	-	20	-	-	1,300	5,400	9,800	0	2,400	-	-
	Settled	-	2.8	$Al_2(SO_4)_3$	50	280	2,300	3,300	1	2,100	-	-
	Conv. Filt.	-	0.2	"	"	10	120	1,700	0	45	-	-
	Dir. Filt.	-	0.2	Polymer	3	50	170	2,700	0	35	-	-
7/11/78	Influent	-	52	-	-	1,300	23,000	20,000	16	2,900	-	$2.5x10^{10}$
	Settled	-	4.6	$Al_2(SO_4)_3$	50	430	6,800	4,700	0	840	-	$2.4x10^8$
	Conv. Filt.	-	0.3	"	"	45	310	1,400	0	40	-	$7.4x10^7$
	Dir. Filt.	-	0.3	Polymer	3	260	5,200	2,400	0	55	-	$5.2x10^7$

TABLE 8 (continued)

Date	Sample	pH	Turbidity (NTU)	Coagulant	Dosage (mg/l)	Indicator Organism Count					Virus Count PFU/ 100 gal.	Seeded MS_2 Phage Count, PFU/ 100 gal.
						Fecal Coli. #/100 ml	Total Coli. #/100 ml	Std. Plate #/ml	Yeast Count #/100 ml	Fecal Strep. #/100 ml		
7/12/78	Influent	-	57	-	-	5,100	33,000	9,400	0	1,800	-	$2.5x10^9$
	Settled	-	6.2	$Al_2(SO_4)_3$	50	900	31,000	7,500	-	7,600	-	$2.9x10^8$
	Conv. Filt.	-	0.3	"	"	260	2,500	2,200	-	85	-	$1.5x10^7$
	Dir. Filt.	-	0.3	Polymer	3	460	5,900	7,500	-	45	-	$2.0x10^7$
7/17/78	Raw River	-	220	-	-	650	50,000	85,000	114	140,000	-	$2.5x10^{16}$
	Influent	10.1	9.0	-	-	0	0	3,000	0	11,000	-	0.0 - Lime Added
	Settled	-	2.5	$FeSO_4$	25	0	12,000	34,000	0	780	-	0.0
	Conv. Filt.	-	0.2	"	"	0	1,500	15.000	0	120	-	0.0
	Dir. Filt.	-	0.2	Polymer	1.5							
7/18/78	Influent	10.0	12	-	-	0	0	1,700	1	2,200	-	$2.5x10^{16}$
	Settled	-	9.4	$FeSO_4$	25	1	15	1,700	0	1,100	-	0.0 - Lime Added
	Conv. Filt.	-	0.3	"	"	0	10	2,700	0	130	-	0.0
	Dir. Filt.	-	0.2	Polymer	1.5	0	0	190	0	45	-	0.0
7/24/78	Raw River	-	-	-	-	26,000	129,000	369,000	735	222,000	-	no seed
	Influent	10.1	13	-	-	0	0	7,400	2	3,400	-	-
	Settled	-	4.7	$FeSO_4$	25	0	1,100	470,000	3	2,300	-	-
	Conv. Filt.	-	0.2	"	"	0	45	8,600	0	120	-	-
	Dir. Filt.	-	0.4	Polymer	1.5	0	75	1,500	0	210	-	-

TABLE 9. REMOVAL OF NATURALLY-OCCURRING MS_2 BACTERIOPHAGE AND ENTEROVIRUS BY DIRECT FILTRATION AND CONVENTIONAL TREATMENT AT LEXINGTON, MISSOURI

Water Sample	Enterovirus* PFU/100 gal. (% Removal)	MS_2 Bacteriophage* PFU/100 gal. (% removal)
Raw Missouri River (3/2/78 to 3/15/78)	7.3	2159
Settled and Filtered (no lime added)	3.2 (56%)	268 (88%)
Direct Filtered (no lime added)	6.1 (17%)	242 (84%)
Raw Missouri River (3/20/78 to 3/30/78)	3.4	1,078
Settled and Filtered (lime added)	1.5 (55%)	110 (90%)
Direct Filtered (lime added)	1.6 (52%)	160 (85%)

*Average number recovered. (Percentage removals based on average numbers recovered.)

TABLE 10. REMOVAL OF TURBIDITY BY DIRECT FILTRATION AND CONVENTIONAL TREATMENT AT LEXINGTON, MISSOURI

Water Sample	Turbidity* NTU (% removal)
[1]Presettled Missouri River (no lime addition)	59.2
Settled and Filtered (no lime addition)	1.6 (97%)
Direct Filtered (no lime addition	4.3 (93%)
[2]Presettled Missouri River (lime added)	65.0
Settled and Filtered (lime added)	3.5 (95%)
Direct Filtered (lime added)	3.1 (95%)
[3]Raw Missouri River	158
Presettled Missouri River	106
Settled and Filtered	1.9 (98%)
Direct Filtered	1.6 (98%)
[4]Presettled Missouri River	40
Coagulated and Settled	8.9 (78%)
Settled and Filtered	0.3 (99%)
Direct Filtered	0.3 (99%)

*Average Value

[1] 3/2/78 to 3/15/78

[2] 3/15/78 to 3/30/78

[3] 4/10/78 to 5/31/78

[4] 7/5/78 to 7/12/78

TABLE II. REMOVAL OF INDICATOR MICROORGANISMS BY DIRECT FILTRATION AND CONVENTIONAL TREATMENT AT LEXINGTON, MISSOURI

Water	Fecal Coliform No/100 ml (% removal)	Total Coliform, No/100 ml (% removal	Std. Plate Count, No/100 ml (% removal)	Yeast, No/100 ml (% removal)	Fecal Strep. No/100 ml. (% removal)
[1]Presettled (no lime addition)	5,100	136,000	42,000	37	149,000
Settled and Filtered (no lime addition)	460 (91%)	13,000 (90%)	6,500 (85)	0.5 (99%)	12,000 (92%)
Direct Filtered (no lime addition)	1,100 (78%)	10,000 (86%)	22,000 (48%)	4 (89%)	23,000 (85%)
[2]Presettled (lime added)	3,400	74,000	77,000	49	290,000
Settled and Filtered (lime added)	0(> 99%)	35 (> 99%)	1,100 (98%)	0 (> 99%)	1,000 (> 99%)
Direct Filtered (lime added)	3 (>99%)	275 (99%)	6,500 (92%)	.25 (99%)	3,400 (99%)
[3]Raw Missouri River	7,000	75,000	60,000	61	180,000
Presettled	3,100 (56)	32,000 (57%)	18,000 (70%)	26 (57%)	73,000 (59%)
Settled and Filtered	190 (94%)	1,500 (95%)	1,100 (95%)	0 (< 99%	1,900 (97%)
Direct Filtered	610 (80%)	5,100 (84%)	3,100 (83%)	0.5 (98%)	6,600 (91%)
[4]Presettled	2,200	18,000	11,000	4	3,200
Settled and Filtered	82	780 (96%)	3,000 (73%)	0 (>99%)	57 (98%)
Direct Filtered	210 (90%)	3,000 (83%)	4,500 (59%)	0 (>79%)	120 (96%)

[1] 3/2/78 through 3/15/78

[2] 3/15/78 through 3/30/78

[3] 4/10/78 through 5/31/78

[4] 7/5/78 through 7/12/78

TABLE 12. REMOVAL OF SEEDED MS_2 BACTERIOPHAGE BY DIRECT FILTRATION AND CONVENTIONAL TREATMENT AT LEXINGTON, MISSOURI

Water Sample	MS_2 Bacteriophage,* PFU/100 liters (% removal)
Pilot Plant Influent (no lime addition)	9.24×10^{9}**
Coagulated and Settled (no lime addition)	1.77×10^{8} (98.1%)
Settled and Filtered (no lime addition)	2.97×10^{7} (99.7%)
Direct Filtered (no lime addition)	2.40×10^{7} (99.7%)
Pilot Plant Influent (lime added)	2.52×10^{16}**
Coagulated and Settled (lime added)	0
Settled and Filtered (lime added)	0
Direct Filtered (lime added)	0

* Average number recovered (percentage removals based on average numbers recovered)

**Calculated value based on kinetic die-away study (Appendix A).

SECTION 7

RESULTS OF STUDIES OF CHLORINE VERSUS CHLORAMINE FOR THE DISINFECTION OF MISSOURI RIVER WATER

INTRODUCTION

The Interim Primary Drinking Water Regulations proposed by the U.S. Environmental Protection Agency in 1976 initiated a controversy over the proposed use of activated carbon for the reduction of trihalomethanes and total organic carbon. One specific subject of debate has been the application of a combined chlorine disinfectant to avoid the formation of trihalomethanes. Although interim regulations permitted the use of "alternate technology" to achieve the 100 ug/l THM limit in place of GAC, new restrictions on the use of alternate disinfectants disallowed the application of combined chlorine residuals in excess of 1 mg/l.

Regulations with respect to the use of chloramine impacted the established practices of numerous water suppliers not originally subject to the proposed organics regulations. Both small and large water utilities which were dependent on river water sources claimed that it was impossible to establish a free chlorine residual. Moreover, these utilities reproached the EPA for a regulation imposed without sufficient data. Water suppliers defended their position with research data indicating that chloramines did, in fact, reduce the formation of THMs. Additionally, decades of public health records were presented to show that no community health problems related to inadequate water disinfection resulted from the use of chloramines.

In late 1978 the U.S. Environmental Protection Agency, MERL, directed an investigation through the University of Missouri-Columbia to obtain in-field data relating to combined chlorine disinfection. The primary objective was to compare the disinfection properties of chlorine and chloramine when applied to conventionally treated Missouri River water. The disinfection capability of each chemical species would be evaluated by comparing the microbial kills at similar points throughout two identical pilot plant water treatment systems.

In addition to the microbiological aspects of the disinfection study, a secondary objective was to collect individual water samples for analyses of trace organic substances. These analyses included: 1) gas chromatography for the determination of trihalomethanes and trihalomethane forming potential, 2) ultra-low level TOC measurement for "purgable" and "non-purgable" organic carbon, and 3) microcoulometric analyses for the evaluation of the carbon adsorbable organic halide (CAOX) compounds. The purpose of the trace organics analyses was to observe the combined effects of disinfection and physicochemical treatment on the formation and removal of naturally-occurring organic materials including TTHM "prerecursors" and total organohalides.

PILOT PLANT OPERATION AND SAMPLING

Figure 29 shows the pilot plant flow and treatment scheme used in the chlorine-chloramine disinfection study. Points 1 through 7 indicate where samples were collected for microbiological and trace organics analyses. Disinfectants were applied by injecting chlorine, as NaOCL, into both pilot plant influent waters just prior to the rapid mix units. Chloramines were formed within one system by adding an ammonium sulfate solution to the river water ahead of the chlorine injection point.

During the six month investigation, pilot plant operations were separated into two phases. Phase I (12/3/78 through 2/23/79) compared bacterial removal between alum-coagulated and alum-coagulated plus chlorine-disinfected river water to obtain baseline data on the effectiveness of physicochemical processes alone with chlorine disinfection. Phase II (4/4/79 through 5/30/79) compared the characteristics of chlorine versus chloramine for disinfection efficiency as measured by the bacterial indicators; total coliforms, fecal coliforms, standard plate counts and yeast organisms. Phase II also facilitated a study of the formation and removal of trihalomethanes from each system by conventional treatment followed by dual media filtration and granular activated carbon post-adsorbers.

Phase I - Microbiological Results

Table 13 presents comparative data obtained using the pilot plant to treat Missouri River water with and without the use of chlorine for disinfection. The results indicate that the average total bacterial removal by physical process (alum-coagulation) alone (Unit #1) was 90% with the majority of the microorganism removal, 80% to 85%, occurring in the sedimentation process. The parallel chlorine-treated system (Unit #2) achieved 99.9% and greater bacterial removals by the end of the sedimentation process.

On days of low influent microorganism populations (e.g., sample date 1/29/79) physicochemical treatment alone produced a finished water which met the suggested bacteriological concentrations for safe drinking water. Additionally, GAC adsorbers in both pilot plant units exhibited no consistent significant influence on the microorganism populations throughout the Phase I operations.

Phase II - Microbiological Results

Table 14 presents the data obtained during the chlorine-chloramine disinfection comparisons. Results indicate that for both pilot plant units; 1) no fecal coliforms or yeast organisms were detectable beyond the influent sampling point, 2) prior to filtration, total coliforms were reduced to levels below the statistical significance of the membrane filter technique, and 3) standard plate counts, after sand/anthracite filtration, were generally reduced to levels approaching the statistical lower limit of testing reliability set at 30 plate counts per ml.

Based on the pilot plant data, a comparison of the indicator microorganism removals between corresponding system sampling points would indicate that chlorine and chloramine are equivalent in disinfecting power.

In addition to the tabulated results, Figures 30 and 31 show levels of standard plate count organisms following settling, dual media (sand/anthracite) filtration and GAC adsorption for chlorine and chloramine-treated systems, respectively. From these

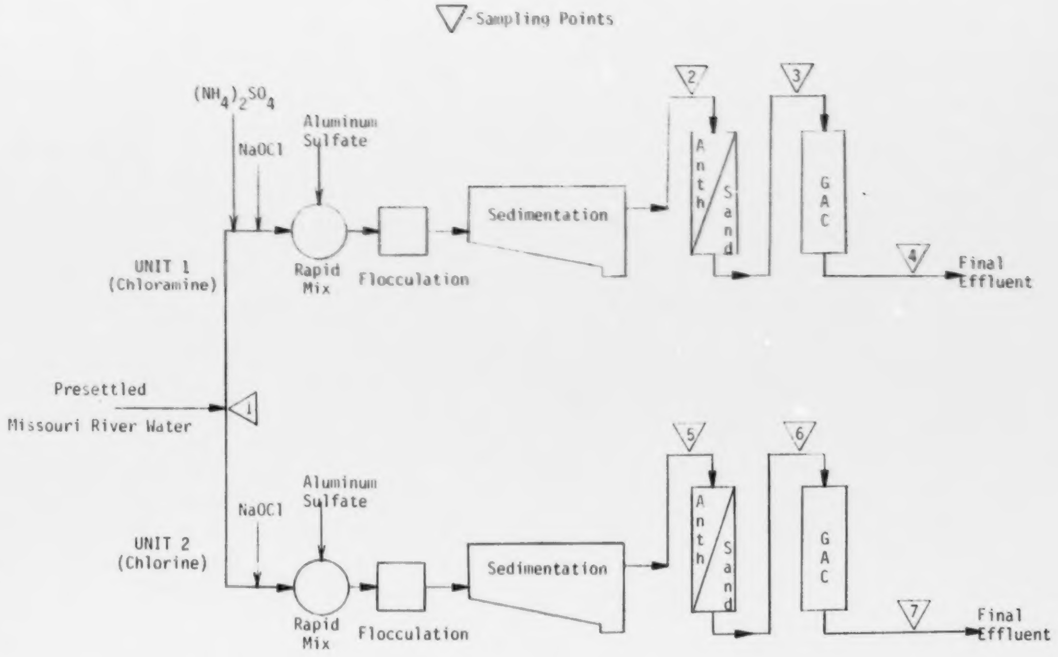
-Sampling Points
$(NH_4)_2SO_4$
NaOCl
Aluminum Sulfate
UNIT 1 (Chloramine)
Rapid Mix
Flocculation
Sedimentation
2
Anth
Sand
3
GAC
4
Final Effluent
Presettled
Missouri River Water
1
UNIT 2 (Chlorine)
NaOCl
Aluminum Sulfate
Rapid Mix
Flocculation
Sedimentation
5
Anth
Sand
6
GAC
7
Final Effluent

Figure 29 Treatment Process Flow Schematic for Missouri River Pilot Plant Disinfection Studies

TABLE 13. PHASE I RESULTS OF MISSOURI RIVER PILOT PLANT STUDIES: PHYSICAL REMOVAL OF MICROORGANISMS, WITH AND WITHOUT CHLORINE DISINFECTANT

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
9/7							
Raw	95,000	5,600	21,000	19	NA	NA	
9/11							
Raw	44,000	4,200	9,200	0	NA	NA	
Presed	20,000	900	3,700	0	NA	NA	
9/17							
Presed	43,000	12,000	38,000	108	NA	NA	
9/18							
Raw	240,000	2,400	23,000	122	NA	NA	
Presed	24,000	6,700	640,000	16	NA	NA	
9/28							
Raw	64,000	5,300	44,000	0	NA	NA	
10/2							
Raw	95,000	2,900	12,000	0	NA	NA	
11/3							
Raw	18,000	9,600	26,000	0	NA	NA	
Presed	120,000	700	46,000	0	NA	NA	
Sludge	25,000,000	75,000	68,000,000	0	NA	NA	
11/15							
Presed	110,000	1,700	6,800	0	NA	NA	
11/17							
Presed	33,000	1,900	92,000	0	NA	NA	

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l)		Turb. (NTU)
					Free	Combined	
11/18							
Raw	150,000	2,700	12,000	8	NA	NA	
Presed	300,000	1,800	9,100	0	NA	NA	
11/20							
Raw	570,000	28,000	39,000	20	NA	NA	
11/29							
Raw	110,000	4,600	53,000	98	NA	NA	
Presed	360,000	1,000	36,000	48	NA	NA	
11/30							
Raw	10,000	6,100	17,000	156	NA	NA	
12/1							
Raw	35,000	2,000	26,000	52	NA	NA	
Presed	680,000	2,600	28,000	56	NA	NA	
12/3							
Presed	930,000	6,200	34,000	4			
Unit 1							
SETT	34,000	170	4,500	0	NA		13
FILT	9,100	350	810	4	NA		1.4
GAC	9,700	250	740	0	NA		0.17
					Cl_2		
Unit 2							38
SETT	10	0	85	0	1.36	0.40	13
FILT	0	0	30	0	1.21	0.28	1.7
GAC	0	0	130	0	NM	NM	0.96

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
12/6							
Presed	320,000	6,000	1,500	0			
Unit 1							
SETT	28,000	570	3,600	0	NA		13
FILT	8,700	130	930	0	NA		1.0
GAC	9,300	180	760	0	NA		0.34
					Cl_2		36
Unit 2					2.38	0.32	
SETT	0	0	0	0	1.88	0.28	13
FILT	0	0	0	0	1.62	0.24	2.3
GAC	0	0	60	0	NM	0.05	0.17
12/10							
Raw	120,000	4,900	3,400	0			
Presed	100,000	4,000	5,400	4			
Unit 1							
SETT	3,200	70	550	0	NA		13
FILT	790	30	120	0	NA		1.5
GAC	50	0	45	0	NA		0.36
					Cl_2		28
Unit 2					2.56	0.44	
SETT	4	0	5	0	1.68	0.42	15
FILT	2	0	2	0	1.48	0.36	1.5
GAC	0	2	6	0	NM	NM	0.39
12/13							
Raw	120,000	2,100	2,000	0			
Presed	12,000	2,400	2,600	0			

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
Unit 1					NA		
SETT	1,800	60	520	0	NA		
FILT	900	20		0	NA		
GAC	1,200	10	60	0	NA		
					Cl_2		
Unit 2					2.16	0.34	
SETT	20	8	1,400	0	1.54	0.28	
FILT	6	0	6	0	1.34	0.26	
GAC	4	0	11	0	0.03	0.02	
12/17							
Raw	91,000	3,100	3,900	0			
Presed	83,000	2,300	3,200	0			
Unit 1	5,300	65	850	0	NA		
SETT	2,200	110	920	0	NA		
FILT	1,100	45	190	0	NA		
					Cl_2		
Unit 2					0.54	0.10	
SETT	270	0	65	0	0.05	NM	
FILT	25	0	60	6	NM	NM	
GAC	0	0	80	0	NM	NM	
12/19							
Raw	93,000	3,400	8,800	8			
Presed	13,00	1,700	4,200	4			

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
Unit 1					NA		
SETT	1,200	35	560	0	NA		
FILT	170	0	60	0	NA		
GAC	180	0	30	0	NA		
					Cl_2		
Unit 2					0.40	0.20	
SETT	32	0	0	0	0.60	0.13	
FILT	16	0	0	0	0.45	0.09	
GAC	0	0	50	0	0.04	0.02	
1/2							
Raw	210,000	4,300	7,700	12			
Presed	310,000	1,700	7,200	10			
1/15							
Raw	2,100	0	340	0			
Presed	2,100	0	890	0			
Unit 1							
SETT	70	0	1,400	0	NA		
FILT	20	0	140	0	NA		
GAC	20	0	35	0	NA		
Unit 2							
SETT	50	0	250	0	NA		
FILT	10	0	25	0	NA		
GAC	0	0	10	0	NA		
1/22							
Presed	250,000	20	2,600	4			

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
Unit 1					NA		
SETT	8,200	0	840	0	NA		
FILT	3,400	8	160	0	NA		
GAC	2,100	0	110	0	NA		
					Cl_2		
Unit 2					0.80	1.92	
SETT	120	0	140	0	0.20	2.15	
FILT	12	0	21	0	NM	NM	
GAC	0	0	26	0	NM	NM	
1/25							
Presed	11,000	100	9,100	16			
Unit 1					NA		
SETT	17,000	23	7,500	6	NA		
FILT	2,300	11	2,700	0	NA		
GAC	1,900	0	1,900	0	NA		
					Cl_2		
Unit 2					0.10	2.00	
SETT	3,000	0	120	0	0.10	2.00	
FILT	25	0	60	0	NM	NM	
GAC	9	0	11	0	NM	NM	
1/29							
Raw	3,600	62	2,700	4			
Presed	4,900	28	1,800	0			
Unit 1					NA		
SETT	170	0	920	2	NA		
FILT	280	0	320	0	NA		
GAC	120	0	220	0	NA		

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
					Cl_2		
Unit 2					0.12	3.03	
SETT	0	0	90	0	0.10	3.00	
FILT	0	0	22	0	NM	NM	
GAC	0	0	22	0	NM	NM	
1/31							
Raw	28,000	110	810	0			
Presed	5,300	23	360	0			
Unit 1					NA		
SETT	1,600	7	290	1	NA		
FILT	630	0	100	0	NA		
GAC	420	4	55	0	NA		
2/5							
Raw	9,600	130	600	8			
Presed	150		280	0			
Unit 1					NA		
SETT	100		100	0	NA		
FILT	35		36	0	NA		
GAC	15		58	0	NA		
					Cl_2		
Unit 2							
SETT	120		12	0	NA		
FILT	7		5	0	NA		
GAC	3		32	0	NA		

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TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
2/7							
Raw	6,400	160	910	10			
Presed	1,100	43	540	10			
Unit 1					NA		
SETT	260	0	200	2	NA		
FILT	78	0	43	0	NA		
GAC	18	0	52	0	NA		
					Cl_2		
Unit 2					0.32	3.69	
SETT	69	0	60	0	0.03	3.12	
FILT	9	0	10	0	NM	2.03	
GAC	4	0	36	0	NM	NM	
2/12							
Raw	10,000		860	4			
Presed	4,600		680	0			
Unit 1							
SETT	710	0	370	0			
FILT	180	0	58	0			
GAC	120	0	51	0			
					Cl_2		
Unit 2					1.03	2.15	
SETT	0	0	56	0	NM	2.00	
FILT	0	0	1	0	NM	1.95	
GAC	0	0	28	0	NM	NM	

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
2/14							
Raw	8,200		700	0			
Presed	90		500	4			
Unit 1							
SETT	410		210	0			
FILT	60		39	0			
GAC	40		45	0			
					Cl_2		
Unit 2					0.40	2.95	
SETT	120		30	0	0.06	2.50	
FILT	8		2	0	NM	2.53	
GAC	2		25	0	NM	0.04	
2/19							
Raw	12,000	200	750	4			
Presed	7,000	50	330	10			
Unit 1							
SETT	270	0	190	0	NA		
FILT	25	0	20	0	NA		
GAC	10	0	28	0	NA		
					Cl_2		
Unit 2					3.0	NM	
SETT	87	0	44	0	NM	NM	
FILT	2	0	4	0	NM	NM	
GAC	3	0	85	0	NM	NM	

TABLE 13 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./100	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)
2/21							
Raw	63,000	600	13,000	31			
Presed	23,000	350	6,200	16			
Unit 1					NA		
SETT	9,100	100	3,400	4	NA		
FILT	2,800	32	1,400	0	NA		
GAC	1,100	12	1,500	0	NA		
					Cl$_2$		
Unit 2					3.0	NM	
SETT	80	0	430	0	NM	NM	
FILT	4	0	88	0	NM	NM	
GAC	8	0	73	0	NM	NM	
2/23							
Raw	780,000	2,700	110,000	240			
Presed	950,000	3,100	100,000	150			
Unit 1					NA		
SETT	210,000	570	34,000	4	NA		
FILT	310,000	600	18,000	1	NA		
GAC	230,000	370	22,000	0	NA		
					Cl$_2$		
Unit 2					2.0	NM	
SETT	260	0	200	0	NM	NM	
FILT					NM	NM	
GAC	66	0	500	0			
2/25							
Raw	1,300,000	1,200	140,000	280	NA		
Presed	820,000	900	130,000	120	NA		

TABLE 14. PHASE II RESULTS OF MISSOURI RIVER PILOT PLANT STUDIES: CHLORAMINE VERSUS CHLORINE DISINFECTION

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./ml	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)	Total Organic Carbon mg/l as C POS*	Total Organic Carbon mg/l as C TOC**	TTHM ug/l as $CHCL_3$	TTHMFP ug/l as $CHCl_3$
4/4											
Raw	82,000	5,400	21,000	200	NA	NH_4^+	190	0.050	6.020	0.7	207.0
Unit 1					NM	2.90	-				
SETT	7	0	270	0	NM	3.17	12	0.005	4.035	1.9	9.1
FILT	3	0	52	0	NM	2.84	6.0	0.007	4.150	lost	lost
GAC	1	0	34	0	NM	0.39	0.7	0.007	3.235	2.1	7.5
					Cl_2						
Unit 2					0.03	2.62	-				
SETT	35	0	250	0	0.03	2.67	14	0.007	4.004	14.9	9.2
FILT	0	0	80	0	NM	2.83	3.5	0.009	4.120	14.9	6.9
GAC	1	0	75	0	NM	0.15	1.3	0.008	3.110	5.8	7.7
4/5											
Raw	72,000	5,500	50,000	44	NA		205				
4/9											
Raw	200,000	4,800	24,000	120	Cl_2	NH_4^+	125	0.002	5.036	0.8	94.7
Unit I					NM	1.36	-				
SETT	10	0	120	0	NM	1.40	6.8	0.003	3.372	2.0	7.7
FILT	0	0	20	0	NM	1.47	1.1	0.004	3.433	1.9	8.2
GAC	0	0	10	0	NM	0.24	0.4	0.002	2.876	2.1	7.1
					Cl_2						
Unit 2					0.03	2.16	-				
SETT	0	0	180	0	NM	1.43	12	0.006	3.980	21.4	10.8
FILT	0	0	36	0	NM	1.43	2.0	0.004	3.927	22.3	9.4
GAC	0	0	64	0	NM	0.09	0.2	0.001	3.077	12.1	6.6
4/10											
Raw	630,000	7,500	74,000	140	NA		125				
4/11											
Raw	250,000	17,000	21,000	240	Cl_2	NH_4^+	125	0.003	5.050	0.5	213.4
Unit 1					NM	1.80	-				
SETT	230	0	100	0	NM	1.62	6.0	0.003	2.567	2.4	7.9
FILT	6	0	12	0	NM	1.75	0.7	0.004	2.578	2.4	7.6
GAC	6	0	16	0	NM	0.21	0.2	0.003	2.162	2.2	7.0
					Cl_2						
Unit 2*					0.25	3.33	-				
SETT	700	0	540	0	0.06	0.85	52	0.010	3.263	50.0	9.2
FILT	750	0	520	0	0.03	0.51	28	0.009	3.233	46.6	20.7
GAC	700	0	770	0	NM	0.16	21	0.005	2.783	27.1	14.1

TABLE 14 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./ml	Yeast No./100	Disinfectant Residual (mg/l)		Turb. (NTU)	Total Organic Carbon mg/l as C		TTHM ug/l as CHCL$_3$	TTHMPF ug/l as CHCl$_3$
					Free	Combined		POS*	TOC**		
4/12											
Raw	60,000	33,000	190,000	570	NA		225				
4/15											
Raw	46,000	4,600	23,000	100	NA		190				
4/16											
Raw	95,000	4,000	25,000	270	Cl$_2$	NH_4^+	140	0.003	4.917	1.0	108.0
Unit 1					NM	1.40	-	-	-	-	-
SETT	30	0	260	0	NM	1.31	14	0.004	3.129	2.2	10.0
FILT	12	0	36	0	NM	1.30	3.0	0.003	2.974	2.0	12.1
GAC	4	0	6	0	NM	0.15	0.35	0.004	2.389	2.5	9.3
Unit 2	6	0	90	0	Cl$_2$ 1.62	1.08	-				
SETT	6	0	90	0	0.27	0.39	-	0.016	3.171	44.1	NM
FILT	3	0	35	0	0.15	0.36	6.0	0.012	3.118	44.2	2.2
GAC	4	0	200	0	NM	0.12	1.7	0.012	2.608	23.1	4.1
4/17											
Raw	80,000	2,700	22,000	29	NA		110				
4/18											
Raw	29,000	2,700	17,000	44	Cl$_2$	NH_4^+	100	0.008	4.275	0.1	101.3
Unit 1					NM	1.84	-				
SETT	3,200	0	170	0	NM	1.78	12	0.011	2.891	2.6	1.7
FILT	0	0	33	0	NM	1.82	2.5	0.011	2.997	2.2	6.7
GAC	0	0	30	0	NM	0.15	0.38	0.010	2.406	2.0	6.1
Unit 2					Cl$_2$ 2.28	0.80	-				
SETT	300	0	30	0	0.54	0.36	10	0.016	3.171	44.1	NM
FILT	0	0	8	0	0.36	0.30	2.5	0.012	3.118	44.2	2.2
GAC	0	0	430	0	NM	0.03	0.43	0.012	2.608	23.0	4.1
4/19											
Raw	43,000	11,000	59,000	64	NA		94				
4/22											
Raw	37,000	3,300	22,000	29	NA		100				

TABLE 14 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./ml	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)	Total Organic Carbon mg/l as C POS*	Total Organic Carbon mg/l as C TOC**	TTHM ug/l as $CHCL_3$	TTHMPF ug/l as $CHCl_3$
4/23											
Raw	300,000	12,000	48,000	230	Cl_2	NH_4^+	180	0.007	5.010	2.2	30.2
Unit 1					NM	1.17	-				
SETT	5,600	0	160	0	NM	1.00	8.7	0.009	3.332	2.1	18.5
FILT	0	0	6	0	NM	1.17	1.2	0.009	3.192	2.0	6.7
GAC	0	0	27	0	NM	0.03	0.29	0.008	2.650	1.9	85.
Unit 2					Cl_2 0.15	0.77	-				
SETT	1,800	0	2,200	0	NM	0.30	120	0.009	4.100	27.1	8.1
FILT	2,000	0	3,300	0	NM	0.27	50	0.009	4.030	23.5	37.4
GAC	1,00	0	1,200	0	NM	0.06	32	0.009	3.547	18.5	38.7
4/24											
Raw	54,000	9,700	51,000	170	NA		180				
4/25											
Raw	70,000	4,900	170,000	55	Cl_2	NH_4^+	280	0.007	4.482	0.3	148.5
Unit 1					NM	1.92	-				
SETT	30	1	170	0	NM	1.27	10	0.011	3.055	1.7	7.4
FILT	0	0	26	0	NM	1.30	1.6	0.011	2.771	1.8	27.1
GAC	0	0	39	0	NM	0.03	0.30	0.009	2.298	2.7	11.2
Unit 2					Cl_2 0.52	0.80	-				
SETT	130	10	900	0	NM	0.09	11	0.011	2.833	10.2	22.6
FILT	50	10	110	0	NM	0.06	1.4	0.001	2.837	10.1	15.3
GAC	55	10	380	0	NM	0.03	0.42	0.009	2.753	13.1	7.0
4/26											
Raw	65,000	4,300	23,000	35	NA		150				
4/29											
Raw	34,000	2,100	18,000	16	NA		100				
4/30											
Raw	78,000	9,000	30,000	46	Cl_2	NH_4^+	150	0.008	4.515	0.4	161.7
Unit 1					NM	1.25	-				
SETT	17	0	130	0	NM	1.17	10	0.011	3.457	1.0	13.3
FILT	0	0	16	0	NM	1.12	1.4	0.011	3.326	2.0	35.6
GAC	3	0	25	0	NM	0.15	0.40	0.010	2.885	2.5	11.9

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TABLE 14 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./ml	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)	Total Organic Carbon mg/l as C POS*	Total Organic Carbon mg/l as C TOC**	TTHM ug/l as $CHCL_3$	TTHMPF ug/l as $CHCl_3$
					Cl_2						
Unit 2					0.15	0.54	-				
SETT	39	0	160	0	NM	0.15	8.9	0.010	3.223	14.9	6.4
FILT	8	0	26	0	NM	0.15	1.0	0.010	3.311	14.1	12.1
GAC	0	0	250	0	NM	0.03	0.29	0.009	2.261	12.7	32.5
5/1											
Raw	150,000	14,000	73,000	130	NA	195					
5/2											
Raw	77,000	11,000	52,000	51	Cl_2	NH_4^+	170	0.006	3.735	0.3	140.1
Unit 1					NM	1.33					
SETT	18	0	280	0	NM	0.12	11	0.008	2.628	2.2	25.8
FILT	0	0	35	0	NM	0.12	2.5	0.009	2.602	2.2	9.3
GAC	0	0	32	0	NM	0.03	0.35	0.008	2.100	2.4	12.8
					Cl_2						
Unit 2					NM	0.63					
SETT	40	0	360	0	NM	0.12	14	0.009	3.134	9.7	14.8
FILT	0	0	87	0	NM	0.12	3.6	lost	lost	9.8	64.0
GAC	3	0	140	0	NM	0.03	0.77	0.008	2.607	11.7	6.5
5/3											
Raw	110,000	9,100	24,000	lost	NA		120				
5/6											
Raw	68,000	2,700	57,000	42	NA		190				
5/7											
Raw	160,000	8,300	67,000	225	Cl_2	NH_4^+	180	0.008	4.215	0.5	185.4
Unit 1					NM	1.71					
SETT	50	0	310	0	NM	1.18	15	0.006	3.635	2.3	13.6
FILT	26	0	60	0	NM	1.07	3.5	0.006	3.617	2.1	13.3
GAC	0	0	51	0	NM	0.15	0.74	0.008	3.070	2.5	9.4
					Cl_2						
Unit 2					0.03	0.54					
SETT	38	0	370	0	NM	0.18	15	0.006	3.497	8.4	18.2
FILT	57	0	150	0	NM	0.15	3.0	0.006	3.447	8.2	59.4
GAC	38	0	150	0	NM	0.03	1.0	0.008	2.920	11.0	21.2

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TABLE 14 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./ml	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)	Total Organic Carbon mg/l as C POS*	Total Organic Carbon mg/l as C TOC**	TTHM ug/l as $CHCL_3$	TTHMPF ug/l as $CHCl_3$
5/8							200				
Raw	140,000	8,000	56,000	85							
5/9					Cl_2		155	0.008	3.745	0.3	166.3
Raw	86,000	5,700	38,000	136	NM	1.48					
Unit 1					NM	0.94	9.7	0.011	2.701	1.6	56.2
SETT	66	0	150	0	NM	0.94	1.4	0.012	2.883	1.8	49.7
FILT	4	0	12	0	NM	0.06	0.25	0.011	2.243	2.8	57.1
GAC	0	0	46	0							
Unit 2					NM	0.51	8.3	0.010	2.721	8.2	43.1
SETT	45,000	0	460	1	NM	0.12	1.4	0.010	2.722	8.5	77.2
FILT	130	0	36	0	NM	0.12	0.25	0.009	2.325	11.1	61.9
GAC	36	0	130	0	NM	0.03					
5/10							120				
Raw	48,000	2,600	17,000	8	NA						
5/13							105				
Raw	46,000	7,800	30,000	12	NA						
5/14					NA		170	0.006	3.745	0.3	166.3
Raw	63,000	5,000	19,000	17	NM	1.24					
Unit 1					NM	1.19	5.6	0.010	2.538	1.5	79.3
SETT	27	0	130	0	NM	1.19	0.43	0.009	2.675	1.6	80.9
FILT	2	0	3	0	NM	0.06	0.15	0.009	2.129	2.6	63.8
GAC	0	0	16	0							
Unit 2					0.90	0.57	8.5	0.014	2.505	38.3	57.4
SETT	27	0	60	0	0.15	0.24	0.92	0.011	2.522	36.3	48.7
FILT	0	0	7	0	0.03	0.21	0.20	0.011	2.165	24.6	51.1
GAC	0	0	160	0	NM	0.06					
5/15							75				
Raw	6,000	3,600	4,800	7	NA						

TABLE 14 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./ml	Yeast No./100	Disinfectant Residual (mg/l)		Turb. (NTU)	Total Organic Carbon mg/l as C		TTHM ug/l as $CHCL_3$	TTHMPF ug/l as $CHCl_3$
					Free	Combined		POS*	TOC**		
5/16											
Raw	44,000	2,200	13,000	2	Cl_2	NH_4^+	89	0.009	3.452	0.3	146.0
Unit 1					NM	1.40					
SETT	0	0	60	0	NM	1.18	4.2	0.010	2.294	1.0	53.3
FILT	0	0	5	0	NM	1.18	0.68	0.010	2.332	1.2	63.3
GAC	0	0	22	0	NM	0.06	0.29	0.010	1.934	1.9	54.7
Unit 2					Cl_2 1.04	0.52					
SETT	0	0	62	0	0.12	0.21	6.5	0.013	2.401	28.3	53.8
FILT	0	0	1	0	0.12	0.21	1.1	0.012	2.345	28.8	51.9
GAC	0	0	68	0	NM	0.06	0.28	0.010	1.980	21.5	41.8
5/17											
Raw	37,000	4,300	38,000	70	NA		150				
5/21											
Raw	3,600	2,700	13,000	2	NA		88	0.001	3.570	0.4	152.0
Unit 1					Cl_2	NH_4^+					
SETT	0	0	290	0	NM	1.19	8.7	0.003	2.611	1.1	61.0
FILT	0	0	27	0	NM	1.15	1.7	0.006	2.655	1.3	78.3
GAC	0	0	27	0	NM	0.06	0.28	0.006	2.203	2.1	69.8
Unit 2					Cl_2 0.36	0.48					
SETT	0	0	120	0	NM	0.24	8.4	0.005	2.402	23.4	54.5
FILT	0	0	36	0	NM	0.21	1.4	0.005	2.391	23.4	56.7
GAC	0	0	1,200	0	NM	0.06	0.19	0.006	2.139	27.4	42.1
5/22											
Raw	22,000	5,000	4,600	0	NA	73					
5/23											
Raw	46,000	4,400	7,700	2	Cl_2	NH_4^+	63	0.003	3.750	0.3	156.2
Unit 1					NM	1.50					
SETT	29	0	53	0	NM	1.42	6.6	0.003	2.772	1.1	78.8
FILT	1	0	3	0	NM	1.33	0.37	0.005	2.696	1.2	80.4
GAC	0	0	52	0	NM	0.06	0.26	0.001	2.274	2.0	73.4
Unit 2					Cl_2 0.87	0.52					
SETT	15	0	51	0	0.06	0.21	5.4	0.004	2.352	24.5	62.6
FILT	16	0	21	0	NM	0.15	1.3	0.002	2.450	24.9	60.0
GAC	0	0	460	0	NM	0.06	0.13	0.002	2.086	19.1	51.3

TABLE 14 (continued)

Sample Date/Type	Total Coliform No./100 ml	Fecal Coliform No./100 ml	Standard Plate Ct. No./ml	Yeast No./100	Disinfectant Residual (mg/l) Free	Disinfectant Residual (mg/l) Combined	Turb. (NTU)	Total Organic Carbon mg/l as C POS*	Total Organic Carbon mg/l as C TOC**	TTHM ug/l as $CHCL_3$	TTHMPF ug/l as $CHCl_3$
5/27											
Raw	58,000	12,000	10,000	4	NA		59				
5/28											
Raw	55,000	7,100	8,100	0	Cl_2	NH_4^+	55	0.003	3.278	0.3	147.2
Unit 1					NM	1.47					
SETT	85	0	110	0	NM	1.30	8.0	0.006	2.542	1.0	80.7
FILT	3	0	14	0	NM	1.28	1.1	0.006	2.467	1.2	60.3
GAC	0	0	250	0	NM	NM	0.17	0.003	2.057	2.7	66.4
Unit 2					1.02	0.43					
SETT	57	0	50	0	0.15	0.21	6.1	0.007	2.469	28.9	71.6
FILT	2	0	8	0	NM	0.18	1.0	0.007	2.370	28.9	48.8
GAC	0	0	350	0	NM	0.16	0.19	0.005	2.035	24.3	60.9
5/29											
Raw	24,000	1,300	4,500	0	NA		55				
5/30											
Raw	28,000	2,600	8,000	1	Cl_2	NH_4^+	50	0.003	3.160	0.2	149.5
Unit 1					NM	1.34					
SETT	0	0	51	0	NM	1.15	5.2	0.006	2.500	1.1	82.3
FILT	0	0	7	0	NM	1.19	1.0	0.006	2.377	1.1	70.3
GAC	0	0	320	0	NM	NM	0.15	0.004	2.036	2.3	45.9
Unit 2					Cl_2 1.28	0.36					
SETT	4	0	20	1	0.24	0.27	3.7	0.009	2.402	36.7	53.6
FILT	1	0	1	0	0.12	0.21	0.78	0.008	2.370	40.2	36.7
GAC	0	0	350	0	NM	0.12	0.20	0.006	1.941	26.1	51.6
5/31											
Raw	18,000	1,200	5,200	7	NA						

figures it would appear that chloramine treatment provided substantial consistency and stability to the bacterial removal within the treatment system. This added stability may be in part due to the less reactive (i.e., oxidative) nature of the chloramines versus the "free" chlorine species. The presence of naturally-occurring ammonia nitrogen (organic nitrogen) in the Missouri River water resulted in the formation of some chloramine in the water treated by chlorine alone.

Standard plate counts in the effluent from the granular activated carbon post-adsorbers were significantly greater than those observed in the filtered water, particularly when chlorine was applied as disinfectant. Increased SPC organism growth was not observed during chloramination until almost 2 months had elapsed.

Phase II - Results of Analysis for Organic Substances

The results of analysis for organic substances in raw and treated Missouri River water are presented in Table 14. The trihalomethane data indicates that chlorine disinfection produces approximately ten times the concentration of THMs as did disinfection with chloramine. In both systems, however, effluent trihalomethane levels were consistently below the proposed 100 ug/1 maximum concentration limit.

Data for the trihalomethane forming potentials (THMFP) indicates that much of the "precursors" to the formation of THMs were removed by the conventional physicochemical treatment. Coagulation/flocculation with aluminum sulfate, followed by settling, generally reduced THMFPs by 45% to 96% based on influent water trihalomethane-forming potentials measured after 48 hours reaction time with 5 mg/1 initial chlorine dosage.

Figure 32 presents plant influent dissolved TOC, plant influent THMFP, and the THMFP for both pilot plant units after settling. Preliminary observations show influent TOC and THMFP to be relatively constant over the two month period. Post-settling trihalomethane forming potentials in both the chlorinated and chloraminated systems exhibit similar trends, gradually increasing over several weeks. Based on the consistency of the influent TOC and THMFP data, it did not appear that a major change in source water dissolved organic species had occurred. Instead, the efficacy of the treatment process may have been decreasing. If so, this loss in ability to remove precursor material may indicate that plant operational factors may strongly influence the removal of naturally-occurring organics by coagulation.

CONCLUSIONS

From the results of the pilot plant studies, the following general conclusions can be stated:

1) Based on standard indicator organism removals, chlorine and chloramine exhibited equivalent disinfecting power over a two month study period.

2) Chloramine produced an order of magnitude less trihalomethanes as compared to chlorine as determined by the liquid-liquid extraction GC technique.

3) Trihalomethane levels in both systems were marginally reduced by GAC filtration over the April-May study period.

4) Trihalomethane "Precursors", as measured by THMFP, were substantially removed by alum coagulation and settling whereas removals on GAC were marginal.

Organism Growth on GAC Post-Adsorbers

Figures 30 and 31 show that standard plate counts in GAC effluents increased beyond the influent levels with time. This coincides with numerous observations of organism growth on granular activated carbon columns both in Europe and in the United States.

In Europe, Ford (20) at Foxcote, U.K., frequently found higher plate counts in the GAC effluent than in the sand filtrate during his pilot plant studies. A five-year comparative study showed 25 percent of the samples of GAC effluent contained more than 500 colonies of bacteria per ml while only three percent of the sand filtrate samples yielded bacterial plate counts in excess of 500 per ml. Ford indentified the predominant organism in the GAC effluent filter as "chlorine-damaged Flavobacteria." The author recommended a 7 day incubation period at 22°C for plate count studies.

A study conducted by Knoppert and Rook (21) at Rotterdam, Germany, indicated that the bacterial quality from a carbon adsorber backwashed daily was slightly better than the effluent from a less frequently washed bed. However, the plate count incubated at 37°C was generally low (less than 10/ml) from either adsorber. This observation implied that the biological growths are not easily removed from the filter column habitat.

In the United States, Sylvia (22) and coworkers investigated the extent of bacteriological regrowth in granular activated carbon filters. Bacterial concentrations (Standard Plate Count at 35°C for 48 hours) were routinely monitored in the applied water and in the effluent from both a 30-inch and 48-inch deep pilot granular activated carbon adsorber. The significant finding was that higher bacterial counts were observed in the effluent samples. Moreover, the organisms that could be identified in the untreated water were not among those organisms that were detected in the GAC effluent. This observation indicated that a natural selection had developed from the heterogenous microbial populations within the water applied to the GAC filter. Consequently, the dominent bacterial group in GAC effluents would not necessarily be the most abundant group of organisms in the applied water.

Hansen, (23) who had several years of operational experience with granular activated carbon adsorbers, recommended that a filter should not be allowed to stand idle for over an hour. He reported bacteriological growth in GAC filters could be controlled through an improved backwashing schedule along with increased chlorination of the applied water.

Other studies have included monitoring of bacteriological development within granular activated carbon beds. At Little Falls, New Jersey, standard plate count and coliform measurements of three full-scale GAC filter effluents were monitored. After

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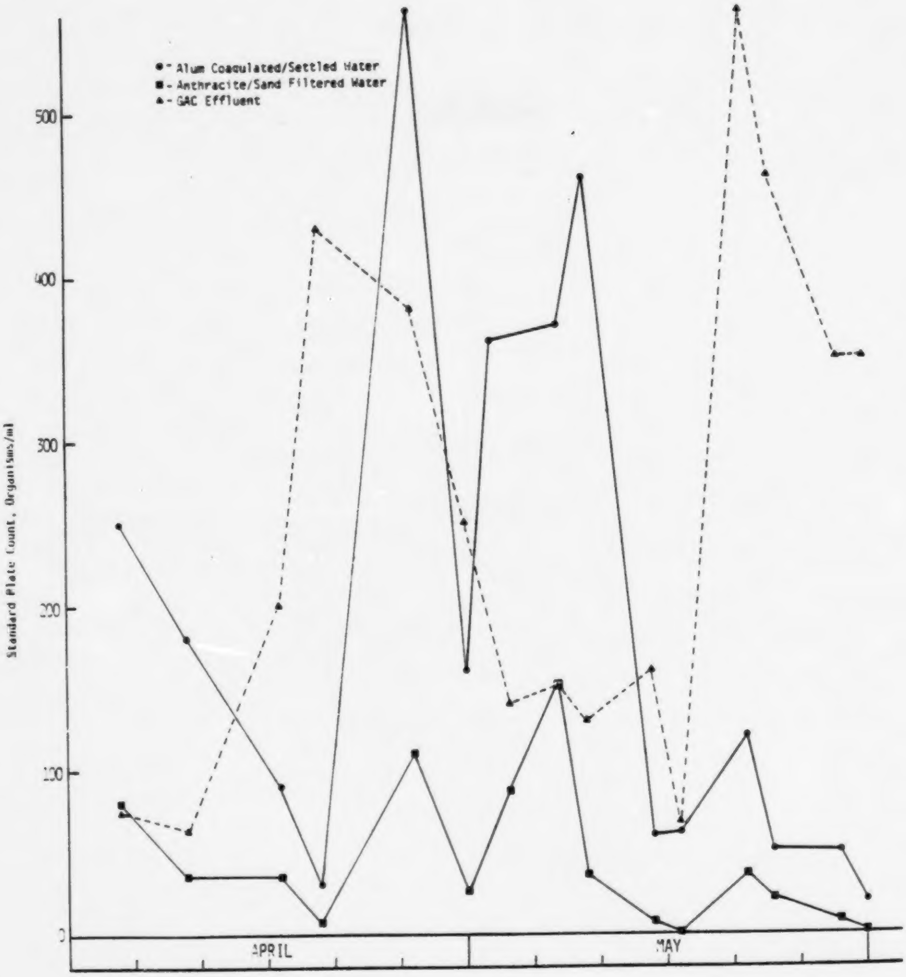

Figure 30 Effect of Chlorination on Standard Plate Count Organism Concentrations

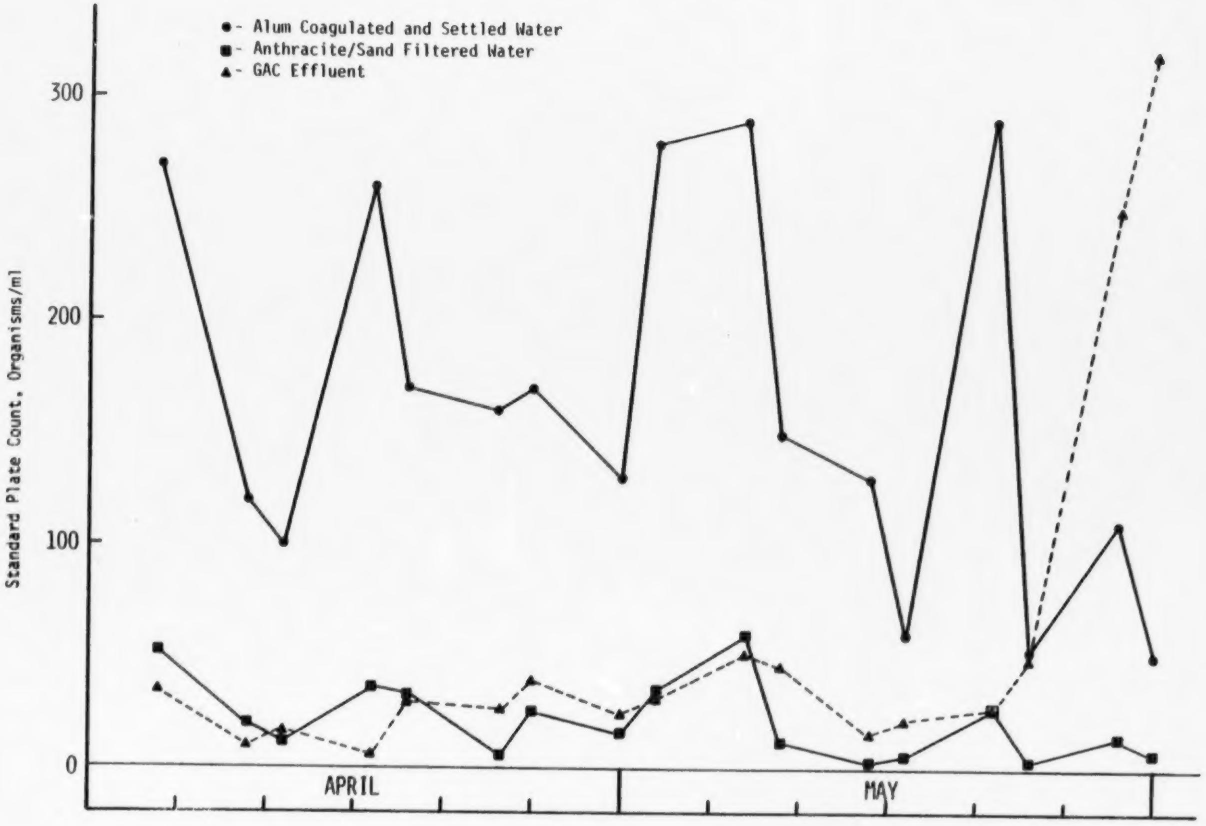

Table 31 Effect of Chloramination on Standard Plate Count Organism

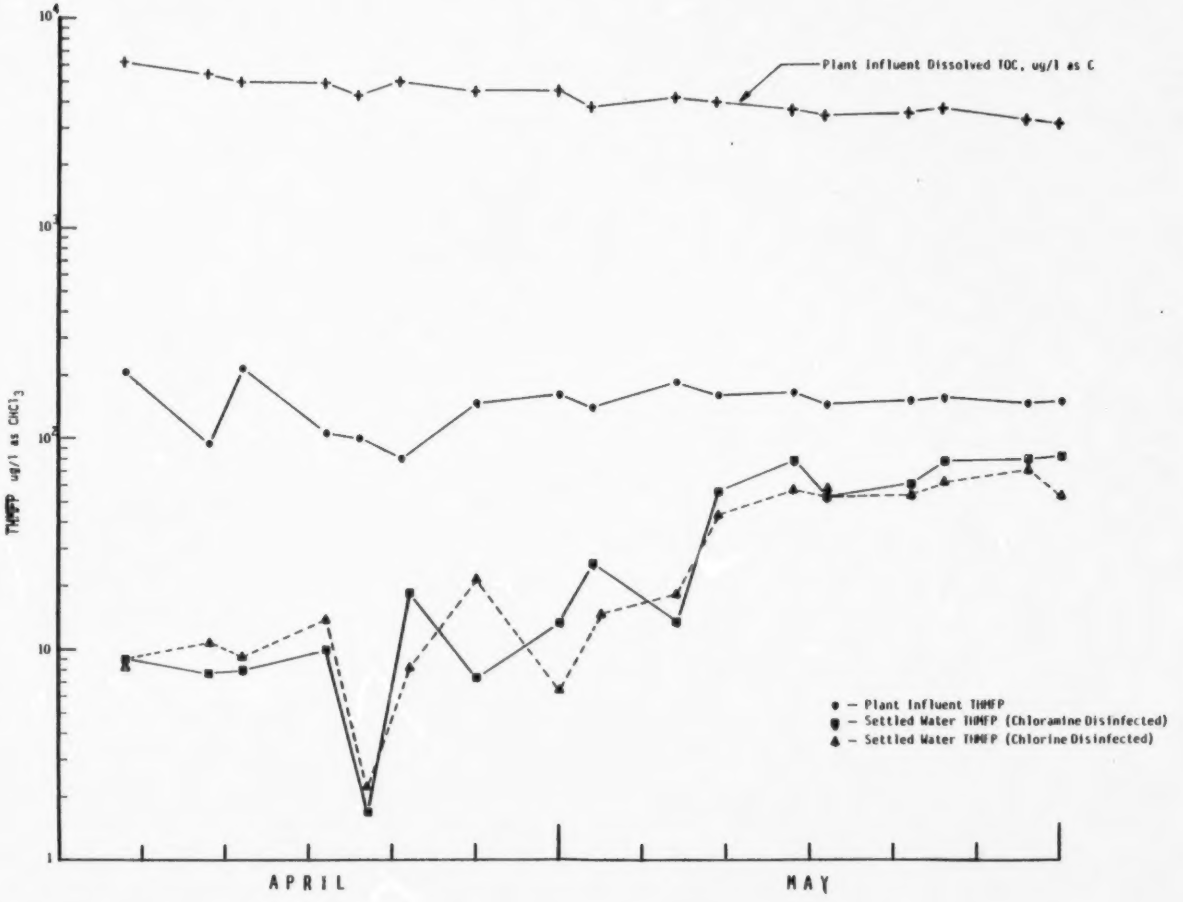

Figure 32 Trihalomethane Forming Potentials for Pilot Plant Treated Missouri River Water

one month in service, the SPC of the GAC effluent was about 100 organisms per ml while that of the applied water was less than 10 per ml. Elevated bacterial concentrations (10^4-10^6 per ml) in GAC filter effluents were also observed by researchers in Columbia, Mo. (O'Connor, et al.) (24) and in Miami, Fl. (Parsons, et al. (25). Parsons examined the effluent from a granular activated carbon column by several isolation methods and concluded that most bacterial growth went undetected when Standard Methods (1) techniques were employed. Some of the organisms identified by Parsons include *Pseudomonas* sp., *Enterobacter agglomerans*, *Acinetobacter*, *Alcaligenes faecalis*, *Moraxella* and *Flavobacterium*.

Much of the controversy in reporting the "total" bacterial count within GAC filters or in drinking water lies in the fact that presently available cultivation methods detect only a portion of the total microorganisms present. To date, no single satisfactory isolation medium is comprehensive for cultivation and enumeration of the microorganism populations in drinking water. Whether the bacterial growth of GAC effluents is observed to be markedly higher than the applied water may also depend on the methods used for bacterial growth and enumeration.

Development of Enumeration Techniques for Organisms from GAC Post-Absorbers

During the pilot plant studies, microbiological analyses were conducted on water samples collected after each process. Since the determination of microorganisms depends on the method of analysis, preliminary studies were directed at the evaluation of cultivation and enumeration methods.

Experiments were conducted to determine the optimum temperature and incubation period for enumeration of microorganisms in water. While the pilot plant experiments were in progress, the bacteriological activity was monitored in terms of the bacterial plate counts on R2A medium* after 7 days of incubation at 25°C. Concurrently, other microbiological analyses on water samples were performed which included standard plate count and the determination of coliform organisms, yeasts and acid-fast organisms.

To further examine the bacterial activity in the GAC adsorption filters, microorganisms that were observed in standard plate count media were randomly selected and isolated for identification purposes. A number of identification procedures were performed to determine cultural and physiological characteristics.

Evaluation of Optimum Cultivation Temperature and Incubation Time

Water samples from the mobile pilot plant at Jefferson City, Missouri were transported to the UMC laboratory for microbiological analysis. Bacterial plate counts were performed on the cultivation media as listed in Table 15. Two temperatures (35°C and 25°C) and two incubation periods (2 days and 7 days) were employed to determine the most satisfactory cultivation technique for the enumeration of bacteria in these samples.

Evaluation of Bacterial Activity in Chlorinated and Unchlorinated Finished Water

Two media, a standard plate count medium diluted to one-half its original concentration (designated at x/2 SPC medium) and R2A medium, were utilized for bacterial plate counts. In addition, two incubation temperatures (35°C and 25°C) and two

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TABLE 15. GROWTH MEDIA EXAMINED FOR ENUMERATION OF MICROORGANISMS IN WATER

1. Standard Plate Count Medium (as Standard Methods)

2. Standard Plate Count diluted one half the designated concentration as in Standard Methods (x/2 SPC)

3. Middlebrooks & Cohn 7H10 Agar plus glycerol

4. Basal Mineral Medium with organic acids

$MgSO_4 \cdot 7H_20$	0.02%
K_2HPO_4	0.1%
$FeSO_4 \cdot 7H_20$	0.005%
$CaCl_2$	0.002%
$MnCl_2 \cdot 4H_20$	0.0002%
$NaMoO_4 \cdot 2H_20$	0.0002%
Na oxalate	0.07%
Na acetate	0.07%
Agar	1%
pH	

5. Trypticase soy agar

6. Polypeptone phosphate agar

7. R2A medium

Yeast extract	0.5g
Proteose peptone No. 3	0.5g
Casamino acids	0.5g
Glucose	0.5g
Soluble starch	0.5g
K_2HPO_4	0.3g
$MgSO_4 \cdot 7H_20$	0.05g
Sodium pyruvate	0.3g
Agar	15g
Dist. or deinized water	1000 ml

Adjust pH to 7.2 with crystalline K_2HPO_4 or KH_2PO_4.

incubation periods (2 days and 7 days) were employed to assess bacterial counts in the final effluents from the two parallel pilot plant treatment systems.

Isolation and Identification of Microbial Populations in GAC Post-Adsorbers

To determine the cultural and physiological characteristics of the microorganisms in the GAC adsorbers, predominant organisms observed in bacterial plate count media were isolated and a number of identification procedures were performed. The major effort was directed toward the development of an isolation medium for the organisms in GAC effluent. The same series of enumeration media listed in Table I6 were examined as candidates for the isolation medium.

Numerous isolates were subjected to extensive subculturing on various media. When a pure culture of an isolate was verified by microscopic and cultural analysis, it was inoculated either into the API identification system or Corning R/B or N/F identification systems.

Results

Evaluation of Culturing Techniques

The results of plate counts of GAC effluent samples on various culturing media are shown in Table I7. In general, the bacterial plate counts on the culturing media at 25°C were higher than those observed on media incubated at 35°C. Moreover, the extended seven day incubation period yielded higher plate counts for all media examined.

In non-chlorinated pilot plant effluents, the bacterial counts on standard plate count medium, by the spread plate method, exhibit plate counts similar to those obtained on R2A medium. In chlorinated pilot plant effluent samples, the final counts (7 days) on Basal Mineral Medium with organic acids were the highest. However, the relative growth efficiencies of isolates observed on R2A medium and x/2 SPC medium were better than on other growth media. Therefore, these two growth media were selected as the enumeration media for bacterial quality of finished water as well for the isolation medium for subculturing microorganisms for subsequent identification procedures.

Evaluation of Bacterial Activity in Chlorinated and Unchlorinated Finished Pilot Plant Water at Jefferson City, Missouri

As expected, bacterial analyses of GAC post-adsorber backwash water yielded high plate counts as shown in Table I7. Again, higher plate counts were observed with extended incubation period. The bacterial analysis of chlorinated effluent showed lower plate counts on both sampling dates. Chlorination appears to control the bacterial growth in both GAC and sand filters, particularly, the latter. The difference may be partly due to the adsorption of the chlorine residual on GAC.

In unchlorinated filter systems, bacterial activities on both the dual media (sand/anthracite) filter and GAC were comparable. Both filters exhibited considerable bacterial growth.

TABLE 16. RESULTS OF BACTERIAL PLATE COUNTS OF GAC EFFLUENT SAMPLES ON VARIOUS CULTURING MEDIA

		Bacterial Plate Counts, No./ml			
		Pilot Plant GAC Effluent* (no chlorination practiced)		Pilot Plant GAC Effluent (Prechlorination practiced)	
		Incubation Temperature		Incubation Temperature	
Media	Incubation Time (days)	35°C	25°C	35°C	25°C
St. Plate	2	45	700	15.5	9
(Spread)	7	130	9600	17.5	Spreader
St. Plate	2	15	305	10	35
(Four)	7	NM	1140	NM	690
St. Plate	2	20	120	5	10
(dilute 1/2)	7	90	980	10	95
(Pour)					
BHM (C)	2	20	90	8.5	0.5
	7	155	3570	36	1540
7H10 Middlebrooks	2	0.5	245	5	1
Cohn with Glycerol	7	9	1898	8	1385
R2A	2	25	300	24.5	10
	7	70	10000	34.5	1025

*Samples derived from operation of mobile pilot plant at Jefferson City, Missouri, January, 1979.

TABLE 17. BACTERIAL PLATE COUNTS IN EFFLUENT AND BACKWASH WATER FROM ANTHRACITE/SAND FILTER AND GRANULAR ACTIVATED CARBON POST-ADSORBERS

Sample/Date	Bacterial Plate Counts, No./ml							
	Unchlorinated System				Chlorinated System			
	Influent water[a]	Effluent water[a]	Backwash Water[b] 2 days	Backwash Water[b] 7 days	Influent water[a]	Effluent water[a]	Backwash Water[b] 2 days	Backwash Water[b] 7 days
Sand Filter								
12/03/78	4500	810	34,000	175,000	85	30	415	470
12/14/78	520	-	219,000	495,000	1,400	6	500	900
GAC Post-Adsorber								
12/03/78	810	720	24,800	109,000	30	130	900	34,000
12/14/78	-	50	75,200	300,000	6	11	1,800	1,800

[a] Plate counts @ 35°C after 48 hours incubation.

[b] Plate counts @ 25°C.

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A compilation of bacterial analyses of pilot plant effluents is shown in Table 18. Again, two incubation temperatures (35°C and 25°C), two incubation periods (2 days and 7 days) and two growth media (x/2 SPC medium and R2A medium) were utilized for bacterial analysis of finished waters from both chlorinated (Unit 1) and initially unchlorinated (Unit 2) systems. Concurrent analyses of standard plate counts, coliform tests, yeasts and acid-fast organisms were conducted to compare the results of bacteriological quality of finished water.

Again, in general, the bacterial plate counts showed consistently higher counts for all culturing media. In addition, 25°C was a better temperature for growth than 35°C. The results indicated that microorganisms can acclimate better in media with lower concentrations of certain complex ingredients and a medium with a variety of organic carbon substances. The growth efficiency of bacteria on R2A and x/2 SPC media was high with up to a three order of magnitude increase in bacterial concentration observed when analyses were performed on these media at 25°C after 7 days of incubation. Usually the R2A medium yielded slightly better growth than the x/2 SPC medium.

As expected, chlorination reduced the bacterial population in both the dual media and GAC effluents. Generally, lower bacterial concentrations were associated with higher chlorine residuals.

The concentrations of microorganisms in both filter effluents (UIE and U2E) were higher than those of influent samples (UIA and U2A), indicating that both dual media and GAC beds were capable of supporting organism growth.

Isolation and Identification of Microbial Populations in GAC Adsorption Filters

In an effort to develop a better subculturing media a number of growth media were examined with isolates obtained from standard plate count media. These growth media included Basal Mineral Medium with two organic acid (oxalate and acetate) supplements; Middlebrook and Cohn Basal Mineral Medium, with and without glycerol; polypeptone phosphate agar; Trypticase Soy agar; Czepalis agar; Standard Plate Count medium diluted one half its normal concentration (x/2 SPC medium) and R2A medium. Each medium supported a fraction of the isolates better than the other growth media examined. Although several media were capable of supporting many isolates to some extent, two growth media (x/2 SPC and R2A medium) were superior for most isolates. These two media were utilized for subculturing into identification media. All isolates were subjected to Gram and oxidase reactions. Additionally, growth characteristics on MacConkey agar and Kligler Iron agar were examined to further aid in isolate identification.

A compilation of cultural characteristics, including Gram reactions and oxidase reactions is presented in Table 19. A number of isolates did not survive and were not available for identification tests. Several isolates were selected for identification by the API identification scheme and the Corning N/F systems.

Specific identifications to the species level were beyond the scope of this research. The data in Table 19 indicate that the isolates were predominantly Gram negative rods, many of which produced pigment. It is likely that many of them are either pseudomonas or florabacteria. Unfortunately, the systems for rapid identifi-

cation of bacteria are oriented toward clinical isolates and identification of isolates such as these is time-consuming and difficult.

TABLE 18. BACTERIAL ANALYSIS OF EFFLUENT FROM GAS POST-ADSORBERS

Date	Sample	Residual Disinfectant mg/l	Total Coliform #/100 ml	Standard Plate Count #/ml	Modified Plate Counts, #/ml			
					2 Day Incubation		7 Day Incubation	
					$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C	$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C
1/15/79	UIE	2.72	0	10	10/5	20/24	95/10	1025/34
	U2E	None	20	35	120/20	300/25	980/90	10000/70
1/24/79	UIE	2.10	9	11	1340/-	778/100	4870/190	1460/300
	U2E	None	1,900	1900		-/-	1.99×10^4/3557	2.22×10^4/3210
1/29/79	UIE	2.15	0	22	54/	36/	2200/	2935/
	U2E	None	120	220	409/	286/	736/	1.57×10^4/
1/31/79	UIE	2.71	3	25	51/37	49/42	2435/60	3020/155
	U2E	None	120	55	404/152	280/211	1335/555	2.56×10^4/3310
2/5/79	UIE		3	32	4/	742/19	64/	940/40
	U2E	None	15	58	382/	674/	478/	768/
2/7/79	UIE	4.00	4	36	5/	50/	1115/	1597/
	U2E	None	18	52	100/	16/	2.18×10^4/	1.41×10^4/
2/12/79	UIE	3.18	0	28	172/	245/	912/	1402/
	U2E	None	120	51	248/	600/	4182/	6960/
2/14/79	UIE	3.35	2	25	/	/	75/	35/50
	U2E	None	40	45	/	/	3487/	1470/100
2/19/79	UIE	3.0	3	85	/	65/	/	1718/
	U2E	None	10	28	10/	20/	2135/	2240/
2/21/79	UIE	3.0	3	73	70/85	55/110	405/110	1175/130
	U2E	None	1,100	1500	3702/1080	3532/1890	7250/1330	6600/2390
2/24/79	UIE	3.0	66	500	/	/538	/	/6500
	U2E	None	2.3×10^5	2.2×10^4	/	/3.67×10^4	/	/4.32×10^4

UIE (Unit 1 Effluent (chlorinated))
U2E (Unit 2 Effluent (Unchlorinated) system effluents)

TABLE 18 (continued)

					Modified Plate Counts, #/ml			
					2 Day Incubation		7 Day Incubation	
Date	Sample	Residual Disinfectant mg/l	Total Coliform #/100 ml	Standard Plate Count #/ml	$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C	$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C
3/24/79	UIA	≃3.0	4	88	100/	120/	110/	140/
	UIE	-	8	73	35/	4.1×10^3/	335/	10^4/
	U2A	none	2,800	1,400	4.3×10^3/	5.3×10^3/	6.3×10^3/	7.7×10^3/
	U2E	-	1,100	1,500	5.7×10^3/	8.2×10^3	1.4×10^4/	1.9×10^4/
4/4/79	UIA	2.84	3	52	50/	50/	45/	220/
	UIE	0.39	1	34	15/	40/	190/	220/
	U2A	2.83	0	80	45/	75/	<10/	200/
	U2E	0.15	1	75	150/	115/	7.4×10^3/	3.8×10^3/
4/9/79	UIA	1.57	0	20	10/40	10/20	120/40	70/20
	UIE	0.24	0	10	4.6×10^3/NM	5.4×10^3/NM	6.8×10^3/NM	9.4×10^3/NM
	U2A	1.43	0	36	20/<10	40/<10	40/<10	300/<10
	U2E	0.09	0	64	200/85	270/20	50/95	330/50
4/11/79	UIA	1.75	6	12	25/50	20/<10	30/50	50/10
	UIE	0.21	6	16	5/5	10/5	15/25	40/15
	U2A	0.51	750	520	200/150	150/350	200/200	500/500
	U2E	0.16	750	770	200/250	320/600	200/250	2.5×10^3/900
4/16/79	UIA	1.30	12	36	40/50	40/50	110/100	150/50
	UIE	0.15	4	6	5/ 10	50/15	120/45	230/75
	U2A	0.36	3	35	100/50	150/<10	150/50	150/100
	U2E	0.12	4	200	360/35	260/60	9.3×10^3/35	3.4×10^4/60
4/18/79	UIA	1.82	0	33	170/150	70/50	3.2×10^3/150	100/50
	UIE	0.15	0	30	270/30	25/10	270/70	200/100
	U2A	0.30	0	9	5/50	5/<10	5/50	5/10
	U2E	0.03	0	430	240/ 10	240/<10	2.9×10^3/500	5.1×10^3/500

UIA (Unit I Influent, chloraminated)
UIE (Unit I Effluent)
U2A (Unit 2 Influent, chlorinated)
U2E (Unit 2 Effluent)

TABLE 18 (continued)

					Modified Plate Counts, #/ml			
					2 Day Incubation		7 Day Incubation	
Date	Sample	Residual Disinfectant mg/l	Total Coliform #/100 ml	Standard Plate Count #/ml	$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C	$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C
4/23/79	UIA	0.77	0	6	<10/<10	<10/<10	30/<10	10/<10
	UIE	0.03	0	27	100/10	20/15	460/10	180/80
	U2A	0.27	2000	3300	400/800	560/900	560/80	1050/$1.6x10^3$
	U2E	0.06	1000	1200	$5.6x10^4$/430	630/350	$8.2x10^4$/430	$1.0x10^5$/350
4/25/79	UIA	1.30	0	26	5/<10	10/<10	20/<10	55/<10
	UIE	0.03	0	39	200/<10	10/10	480/85	390/100
	U2A	0.06	50	110	200/50	80/ 10	420/300	400/200
	U2E	0.03	55	380	810/110	85/30	$2.6x10^4$/750	$5.6x10^3$/$1.2x10^3$
4/30/79	UIA	1.12	0	16	10/<10	25/<10	55/<10	110/<10
	UIE	0.15	3	25	10/25	<10/20	430/125	890/160
	U2A	0.15	8	26	160/50	260/<10	110/50	360/50
	U2E	0.03	0	250	$1.3x10^4$/130	$9.8x10^3$/145	$2.5x10^4$/$4.2x10^3$	$5.7x10^4$/$3.7x10^3$
5/3/79	UIA	0.12	0	35	15/<10	25/<10	95/<10	100/<10
	UIE	0.03	0	32	5/<10	<10/<10	490/<10	450/<10
	U2A	0.12	0	87	160/<10	250/100	160/<10	430/100
	U2E	0.03	3	140	$1.2x10^4$/$1.6x10^4$	$2.1x10^4$/600	$4x10^4$/$1.6x10^4$	$2.8x10^4$/600
5/14/79	UIA	1.19	2	3	60/100	60/<10	70/150	85/50
	UIE	0.06	0	16	60/60	40/45	$3.1x10^3$/410	$1.1x10^3$/510
	U2A	0.21	0	7	250/300	150/250	600/450	350/350
	U2E	0.06	0	160	$1.2x10^3$/200	170/400	$3.4x10^4$/$1.6x10^3$	$9x10^3$/$2.9x10^3$
5/16/79	UIA	1.18	0		5/<10	5/<10	15/<10	5/<10
	UIE	0.06	0		25/20	35/<10	306/365	730/355
	U2A	0.21	0		15/<10	50/<10	15/<10	10/50
	U2E	0.06	0		750/50	260/ 10	$3.7x10^4$/$2x10^3$	$1.2x10^4$/$1.6x10^3$

TABLE 18 (continued)

					Modified Plate Counts, #/ml			
					2 Day Incubation		7 Day Incubation	
Date	Sample	Residual Disinfectant mg/l	Total Coliform #/100 ml	Standard Plate Count #/ml	$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C	$\frac{X}{2}$ 25°C/35°C	R2A 25°C/35°C
5/21/79	UIA	1.15	0	27	20/35	10/5	20/35	40/15
	UIE	0.06	0	27	10/<10	15/5	3.3x10^3/450	2.2x10^3/600
	U2A	0.31	0	36	10/<10	35/100	30/100	45/100
	U2E	0.06	0	1,200	3.6x10^3/2.7x10^3	2.4x10^3/2.4x10^3	5.2x10^4/6.4x10^3	3.2x10^4/4.8x10^3
5/24/79	UIA	1.33	1	8	<10/5	5/<10	20/20	10/<10
	UIE	0.06	0	52	10/5	20/75	3.7x10^3/200	3.9x10^3/720
	U2A	0.15	16	21	20/<10	5/5	25/<10	35/10
	U2E	0.06	0	460	2.5x10^3/2.3x10^3	2.1x10^3/1.3x10^3	4.1x10^4/3.2x10^3	3.6x10^4/3.2x10^3
5/28/79	UIA	1.28	3	14	10/5	10/10	20/15	25/15
	UIE	NM	0	250	150/15	50/<10	4.9x10^3/1.9x10^3	4.2x10^3/1.1x10^3
	U2A	0.18	2	8	5/50	<10/50	40/50	20/50
	U2E	0.06	0	350	3.7x10^3/1.8x10^3	3x10^3/3.8x10^3	2.7x10^3/3.5x10^3	1.8x10^4/5.6x10^3
5/30/79	UIA	1.19	0	7	10/10	10/5	50/10	50/10
	UIE	NM	0	320	<10/25	100/180	4.2x10^3/1.6x10^3	2.5x10^3/1,2x10^3
	U2A	0.33	1	1	15/<10	60/ 10	45/<10	60/50
	U2E	0.12	0	350	4.6x10^3/<10	4.6x10^3/2x10^3	5.2x10^4/3.4x10^3	1.9x10^4/6x10^3

NM - Not Measured

EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
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TABLE 19. CULTURAL CHARACTERISTICS OF MICROORGANISMS OBSERVED IN FILTER BACKWASH AND EFFLUENT WATERS FROM THE MOBILE WATER TREATMENT PILOT PLANT AT JEFFERSON CITY, MISSOURI

Isolate number	Cellular Morphology	Pigment Production on R2A or x/2 SPC medium	Subculturing Medium Preference[a]	Gram Reaction[b]	Oxidase Reaction[c]	Growth on[a] R2A Medium without glucose	Growth on[a] Kligler Iron Medium	Growth on[a] MacConkey Agar Medium	Comments
Chlorinated Dual Media Filter									
A-1	curved rods	NP	x/2 SPC	-	-	+	++	+	
A-2	small thin rods	yellow (diffusible brown)	x-2 SPC	-	-	+	++	+	
A-3	small rods	NP	R2A	-	-	++	++	+++	
A-4	small rods in chains	yellow	x/2 SPC	-	-	++	++	+	
A-5	cocci	yellow mucoid	x/2 SPC	+	-	++	+++	+	
A-6	tapered end rods	yellow	x/2 SPC	-	-	+++	+++	+++	
A-7	rods	yellow	x/2 SPC	±	-	++	++	+++	
A-8	small rods	NP	R2A	-	+	+	+++ (black butt)	+++	
Chlorinated Dual Media Filter Effluent									
B-1	slightly curved rods	NP (diffusible green)	x/2	-	+	+	+++ (black butt)	+++	
B-2	cocci	NP	x/2	+	+	++	+++	++	
B-3	very small rods	NP	R3A	-	-	-	-	-	motile
B-4	coryneform	yellow	R2A	+	+	++	+	+	
B-5	rods	NP	(Lost during subculturing)						
B-6	long, thin	NP		+	+	++	+++	++	
B-7	small rods	NP	R3A	+	-	+	+	++	spore-former
B-8	small, curved rods	yellow	x/2 SPC	-	-	++	+++	+++	motile
B-10	rods	NP to light tan	(Lost during subculturing)						
B-11	rods		x/2 SPC	+	=	+	++	+	
B-12	small rods	NP	x/2 SPC	+	=	+	++	++	
B-13	rods	NP	R2A	+	=	+	++	++	motile

TABLE 19 (continued)

Isolate number	Cellular Morphology	Pigment Production on R2A or x/2 SPC medium	Subculturing Medium Preference	Gram Reaction	Oxidase Reaction	Growth on R2A Medium without glucose	Growth on Kligler Iron Medium	Growth on MacConkey Agar Medium	Comments
B-14	short rods	NP	R2A	v	-	+	++	+	
Unchlorinated Anthracite Filter Backwash Water									
C-1	short rods	purple	x/2 SPC	-	-	++	+++	+++	
C-2	coccobacilli	yellow	R2A	+	-	++	+++	+	
C-3	ellipsoidal rods	light yellow	R2A	v	-	+++	+++	+++	mucoid colony
C-4	rods	NP	R2A	+	-	++	+++	++++	mucoid colony
C-5	small rods	yellow	R2A	+	-	++	+	+	
C-6	bacteroid	yellow	R2A	+	+	+++	++	-	
C-7	ellipsoidal rods	yellow	x/2 SPC	-	-	+	+	+	
C-8	coccobacilli	yellow	SPC	(lost during subculturing)					
C-9	polymorphic shapes	NP	R2A	-	-	+	-	+	mucoid colony mucoid colony
C-10	long rods	tan	R2A	-	-	+	+++	++	
C-11	cocci	NP	R2A	v	-	+	+++	+++	
c-12	coccobacilli	NP	R2A	v	-	+	+	+	diffusible brown pigment
C-13 to C-15	all rods	all NP	none	(Lost during subculturing)					
C-16	thin rods	NP	x/2 SPC	-	+	+	++	++	
C-17	small rods	NP	R2A	-	+	+++	+++	+++	green diffusible pigment
C-18	oval rods	NP	(Lost during subculturing)						
C-19	short rods	NP	(Lost during subculturing)						
C-20	short rods	NP	(Lost during subculturing)						
C-21	short rods	yellow	(Lost during subculturing)						
C-22	long, thin rods	yellow	SPC	-	-	+	++	++	
C-23	cocci	yellow	(Lost during subculturing)						
C-24	ellipsoidal rods	NP	R3A	v	-	+	-	+	
C-25	cocci	NP	(Lost during subculturing)						

TABLE 19 (continued)

Isolate number	Cellular Morphology	Pigment Production on R2A or x/2 SPC medium	Subculturing Medium Preference	Gram Reaction	Oxidase Reaction	Growth on R2A Medium without glucose	Growth on Kligler Iron Medium	Growth on MacConkey Agar Medium	Comments
C-26	cocci	yellow	(lost during subculturing)						
C-27	short rods	yellow	(lost during subculturing)						
C-28	coccobacilli	NP	(lost during subculturing)						
C-30	rods	NP	SPC	+					spore-former
C-31	short rods	yellow	R2A	-	+	++	+	-	
C-32	bacteriods	pink	R2A	-	-	+	-	-	mucoid colony
C-33	coccobacilli	NP	x/2 SPC	-	-	+	-	-	
C-34	rods	pink	R2A	-	-	+	-	-	
C-35	rods	orange	R2A	v	-	+	-	-	
C-36	short rods	NP	R2A	-	-	+	+	+	
C-37	small rods	orange	R2A	-	-	+	+	+	
C-38	rods	pink	R2A	-	+	+	+	+	
C-39	large rods	yellow	R2A	-	-	++	-	-	
C-40	small rods	NP	R2A	-	+	+	+	+	mucoid colony
C-41	small rods	pink	R2A	-	-	+	+	+	
C-42	very small rods	pink	R2A	-	-	+	+	-	
C-44	cocci	NP	R2A	-	-	++	+	+	
Unchlorinated GAC Effluent									
D-1	short rods	NP	R2A	+	-	+	++++	+++	
D-2	coccobacilli	NP	R2A	+	-	+	+++	+++	
D-3	tapered rods	yellow	R2A	-	(Not Available)				
D-4	bacteroids	yellow	x/2 SPC	v	+	+	-	+	
D-5	tapered rods	pink	x/2 SPC	-	-	+	++	+	
D-6	vibrio shaped	NP	R2A	+	-	++	+	++	
	rods								
D-7	rods	NP	R2A	+	NA	NA	+	++	diffusible pigment
D-8	rods	pink	x/2 R2A	v	-	-	-	+	
D-9	coccobacilli	NP	R2A	+	+	++	+++	+++	
D-10	rods	light yellow	(lost during subculturing)						
D-11	thin rods	light orange	(lost during subculturing)				+		
D-12	rods	yellow	R2A	v	-		+	+	

TABLE 19 (continued)

Isolate number	Cellular Morphology	Pigment Production on R2A or x/2 SPC medium	Subculturing Medium Preference	Gram Reaction[b]	Oxidase Reaction[c]	Growth on R2A Medium without glucose	Growth on Kligler Iron Medium	Growth on MacConkey Agar Medium	Comments
D-13	small rods	NP	R2A	-	+	+	+	+++	fluorescent pigment
D-14	rods	yellow	(lost during subculturing)						
D-15	short rods	NP	(lost during subculturing)						
D-16	short rods	NP	None	-	-	-	-	++	
D-17	rods	yellow	SPC	-	-	+	-	+	
D-18	cocci	NP	None	+	-	+	-	+	
D-19	very thin rods	NP	(lost during subculturing)						
D-20	rods	yellow	(lost during subculturing)						
D-21	rods	NP	(lost during subculturing)						
D-24	ellipsoidal rods	NP	None	-	NA	NA	+	+	
D-25	rods	pink	R2A	-	+	++	+	+	
D-27	coryneform	yellow	R2A	+	-	++	+	+	
D-28	long rods	NP	R2A	-	-	+	++	+	
D-29	small ellipsoidal rods	NP	R2A	-	+	+	++	+	
D-30	rods	NP	R2A	-	-	+	+	+	

[a] x/2 SPC: Standard Plate Count Medium diluted one-half of Original Concentration
R2A: Reasoner and Geldreich Medium

[b] +: Gram positive
-: Gram negative

[c] +: Oxidase positive
-: Oxidase negative

[d] -: No growth
+: Scanty growth
++: Fair Growth
+++: Good Growth
++++: Excellent Growth

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APPENDIX A

VIRUS CONCENTRATION AND ASSAY PROCEDURES*

I. INTRODUCTION

The AQUELLA$^{\text{TM}}$ Virus Concentrator is an apparatus which provides the capability of isolating small numbers of viruses from large volumes of water and delivering them in concentrated form for laboratory assay. The apparatus consists of a number of parts which may be interconnected in any of several ways to carry out various unit operations either singly or in continuous sequences. The arrangement of parts and the choice of unit processes depends on a number of variables such as the chemistry of the water being sampled, the size of the sample being taken, the type of viruses being sought, and several other factors which will become apparent later.

The AQUELLA$^{\text{TM}}$ Virus Concentrator consists of a portable container which acts as a support for the component apparatus. These components consist of an electrical water pump, by-pass valve, purge line and safety valve followed by a series of three filter holders and a flow meter. There are containers to hold the adsorbing filters and to deliver the eluting solutions. A small compressor provides the means of propelling solutions and of removing residual liquids from the system.

Additionally, there are assorted connecting hoses, a pH meter, and a plate filter holder.

Typically, the sequence of operations would be as follows. Water would be pumped from the source of supply through the three filter holders and the flow meter. After this removal of particulates, the water would pass through the water actuated pump which would, by means of satellite pumps, inject acids and salts to modify its chemistry. It would then pass through the static mixer, the water meter, a wound adsorbing filter placed in the final filter holder and finally, through a plate filter held in the stainless steel filter holder.

The unit operations will be covered in detail later.

The components are contained or supported by a hinged carton, which provides a means of transporting and supporting the component parts as a unit.

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*Prepared by the Carborundum Corporation, R&D Water Management Division, Niagara Falls, New York

II. PREPARATION

Prior to the beginning of any sampling experiment, a number of preliminary operations should be undertaken.

A. Cleaning

Each of the component parts of the apparatus should be well cleaned and copiously rinsed with clear water to remove any traces of residual detergents or other contamination.

B. Assembly

The component parts should then be connected in the sequence decided upon for the experiment. Following this, it should be briefly rinsed with clear water, to insure the absence of leaks and show the proper connection of component parts.

C. Sterilization

The apparatus should be filled with dilute acid (pH 0.5-1.0) and allowed to stand for thirty minutes. All filters should be in place for this procedure. Pump the sterilizing solution through the rest of the unit. This is done to insure that no surfactant or sizing from the clarifiers comes into contact with the adsorbants. The rinse should be done in the same order.

After the prescribed contact period the unit should be rinsed with water to be sampled until the effluent pH reaches that of the influent water. It may be necessary to place an extra filter ahead of the pump for the entire operation (sterilizing and rinsing) to prevent the clarifiers from becoming plugged during the rinse. This filter should be removed before sampling begins. It is desirable to flush the exterior of the filter holders and quick connect fittings in a similar way to minimize the chance of contamination during subsequent operations.

The individual filters can safety be autoclaved without adversely affecting their subsequent behavior.

DO NOT AUTOCLAVE THE FILTER HOLDERS OR HOSES.

D. Sample Size

The calculations and solution preparations are based on the study of 378 liters (100 gallons) of water. It is not necessary, or indeed practical to process such large volumes of water in many cases. The sample size chosen should only be large enough to yield statistically valid results. A dirty water, such as a sewage effluent for example, will contain both organics and viruses in concentrations sufficiently high to provide the necessary numbers of virus isolates from a few gallons. To attempt to process large volumes such as 378 liters (100 gallons) will like present operating problems due to the formation of filter plugging flocs and the depletion of virus adsorbing sites. The latter will lead to lower virus recovery efficiencies and might infact lead to erroneously negative results. To establish this limit, it is necessary to run a seeded experiment taking grab samples of effluent at ten gallon intervals, and assaying to determine the volume at which the recovery efficiency drops. A standard sample size will be the largest volume before recover rate drops.

E. Water Chemistry

1. Chlorine

The water to be tested should be tested for active chlorine residual. An O-Tolidine kit is suitable for this. If the possibility exists that chlorine might be present during the period of the test, it is desireable to prepare a solution of sodium thiosulfate ($Na_2S_2O_3$) as described in a later section and inject this continuously throughout the experiment. A small residual sodium thiosulfate concentration has no known adverse effects on virus concentration.

2. Acid Capacity

If it is decided to carry out the virus concentration stepwise by the batch method, instead of using the continuous injected method*, this step is not necessary. If continuous injection method is to be used, proceed as follows:

Titrate 380 ml of water to be studied to pH 3.5 using 0.12 molar hydrochloric acid (0.12 M HCl), and note the volume required. Add 11 times this volume of concentrated hydrochloric acid (12 M HCl) to 20 liters of distilled or deionized water in the container in which the injecting salt solution is being prepared.

F. Solutions

1. $AlCl_3$/HCl Injecting Solution

On the basis of the size of the sample to be taken, prepare 20 liters of hundredth molar aluminum chloride (0.01 M $AlCl_3$) for each 378 liters (100 gallons) of water to be sampled.

Add 48.3 grams of hydrated aluminum chloride ($AlCl_3 \cdot 6H_2O$; molecular weight 241.43) to twenty liters of distilled or deionized water** in a large plastic container. WARNING: DO NOT USE ANHYDROUS ALUMINUM CHLORIDE. HEAT OF HYDRATION WILL CAUSE VIOLENT SPATTERING.

When injected at a ratio of 1:20 into the sample water, the final pH of 3.5 and concentration of 0.0005M aluminum will result.

2. Thiosulfate Solution

By means of an O-Tolidine test kit, or other standard method, determine whether active chlorine compounds are present in the water to be sampled If the results are positive, prepare 4 liters of sodium thiosulfate solution. Use 32 grams of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$; molecular weight 248.18) for each ppm of chlorine found to be present. Distilled or deionized water should be used to make the solution. The solution is added at the rate of 1 part per 100 by means of the proportioning pump, using the smaller injector. A setting of 1.3 (oz./gal.) will deliver 1%. A slight excess of thiosulfate will in no way adversely affect the concentration of viruses.

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* For batch method, see section 4B.

**Use only distilled or deionized water to prepare solutions to avoid unanticipated reactions, precipitates, etc., and possible damage to apparatus.

3. Alkaline Glycine

Prepare 0.10 molar Glycine (NH_2CH_2COOH; molecular weight 75.07) solution by dissolving 37.50 grams of glycine in 5 liters of distilled or deionized water. Adjust to pH of 11.5 by titrating with ten molar sodium hydroxide (10M N_aOH; molecular weight 40) prepared by dissolving forty grams of sodium hydroxide in 100 ml of distilled or deionized water. CAUTION: Dissolve the sodium hydrozide slowly in small portions to prevent the solution from becoming excessively hot. Glycine solutions should not be stored for an extended period since they are somewhat unstable and the pH shifts on storage. Refrigerate them until use. Check the pH immediately before use to be certain that a pH of 11.5 is maintained.

4. Acidic Glycine

Prepare a 0.10 M glycine (NH_2CH_2COOH; molecular weight 75.07) solution by disolving 20.56 grams of glycine in 5 liters of distilled or deionized water. Adjust to pH 2.0 by titrating with concentrated hydrochloric acid (12M HCl). Keep the solution refrigerated and do not store for a prolonged period. Check the pH immediately before use.

5. Acidic Saline

Prepare a saline solution by dissolving 42.5 grams of sodium chloride (NaCl, molecular weight 58.44) in 5 liters of distilled or deionized water. Adjust the pH to 3.5 by titrating with one molar hydrochloric acid (1.0M HCl). This salt concentration is approximately isotonic for human enteric viruses.

6. $AlCl_3$ Stock

Prepare 100 ml of three tenths molar aluminum chloride (0.3 M $AlCl_3$) by dissolving 7.23 grams of hydrated aluminum chloride ($AlCl_3 \cdot 6H_2O$; molecular weight 241.43) in 100 ml distilled or deionized water.

7. Phenol Red

Prepare a 0.1% solution of Phenol Red indicator by dissolving 0.1 gram of Phenol Red (sodium salt) in 100 ml ofdistilled or deionized water. It may be necessary to add a little 1 N NaOH to get the salt to dissolve.

8. Sodium Carbonate

Prepare a 1 M solution of sodium carbonate (Na_2CO_3; molecular weight 105.99) by adding 10.6 grams to 100 ml distilled or deionized water.

9. Fetal Calf Serum

Fetal calf serum should be heat inactivated by placing it in a 56°C water bath for 30 minutes. The serum should then be aseptically aliquoted into 5-10ml volumes and frozen until ready for field use.

G. Field Preparation

1. Detergent Soaking

The No. 3 filter will, if not treated, remove viruses under certain conditions. To eliminate this possibility, the No. 3 filter should be soaked in a 0.1% solution or surfactant* for a minimum of one hour, then washed until no further evidence of foaming is detected.

As prepared, textile filters contain sizing and lubricating compounds added to enhance their spinning properties. Under certain conditions, these may be toxic to certain cells, and may interfere with virus recovery.

2. Washing

All of the clarifying filters can be washed in place by pumping filtered water through them (use a #4 wound filter), being certain that the wash water is discharged to drain and does not contact either of the virus adsorbing filters.

A better method, and one that saves time in the field at the sampling site, is to prepare a series of two (or more filter holders in the laboratory. In the first, place a No. 4 filter, and in the second (and following) holder(s) the clarifying filters. They can be washed using tap water and line pressures. A few gallons of water is adequate to remove sizing compounds. Upwards of fifty gallons may be required to free the third clarifying filter of traces of detergent. Removal of final traces is greatly facilitated by repeatedly blowing the filter "air dry" of excess water after ten to fifteen gallon washes.

3. Autoclaving

Each of the first three clarifying filters can be sterilized by autoclaving without adverse effects. Do not autoclave filter holders, or hoses, except the stainless steel virus adsorbing plate filter. The plastics used throughout the system should be sterilized by means of extreme pH treatment.

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*Tween 80 (polyoxyethlene (20) sorbitan monooleate)
Obtained from: The Carborundum Company
R & D/Water Management - P.O. Box 1054
Niagara Falls, NY 14302

III. OPERATIONS

Each operation in the system is covered separately. They may be used in a variety of sequences, which will be described later, depending on the quality of the water being tested, the size of the sample and the procedure being used.

A. Pumping

Each unit is equipped with a rubber impellor pump, rated to deliver up to 80 gal/hr at 40 psig. Care should be taken never to operate pump when dry, or to allow it to ingest abrasive particles. Either of these will drastically reduce its efficiency, and shorten its life. The rubber impellor pump has a grease fitting which should be used periodically to lubricate it. A copy of the operating instructions for the pump is included. It should be read before the pump is used.

B. Clarification

The apparatus is provided with three holders in series to hold fiber wound clarifying filters. In usual operations, these filters are 5 micron, 1 micron and 1 micron, the last filter being present to remove by adsorption certain materials which might otherwise interfere with virus recoveries. This choice of filters represents a good starting point, which may be modified to meet specific problems. For example, a very turbid water, or one carrying a high solids load might better be handled with a 100 micron, 20 micron, 1 micron filter series, while a very clean water such as a finished tap water might require only a single clarification filter before the virus adsorption step. Before use, the clarifying filters should be treated as described in Sections IGI-3.

The clarifying filters are supported in see-through filter holders. This provides a visual indication of possible plugging and flow restriction and suggests whether elution from them might be warranted to examine for possible viruses adsorbed on the particulates.

C. Chemical Adjustment

In order to maximize virus adsorption, the chemistry of the water is modified. The pH is lowered to 3.5 by means of acid addition and a polyvalent salt is added, 0.0005 M Al. Solutions are added by means of the chemical injector in the continuous mode of operation or they are added batchwise to the clarified water if the batch method of operation is used.

In usual operation, each of the two larger satelite pumps on the injector is set at 3.2 (oz/gal) and they are used to feed the $AlCl_3$ - HCl solution into the clarified water stream continuously.

Should the test water be found to contain residual chlorine, the third satellite pump is used to inject a thiosulfate solution, typically using an initial setting of 1.28 (oz/gal).

Prior to diverting the chemically adjusted water stream to the adsorbing filters, it is diverted to drain and samples of effluent water of one liter are taken to test the pH and to determine whether the chlorine residual has been eliminated. When such tests

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show that the proper chemistry has been achieved and the system is stabilized, the water stream is diverted to the adsorbing filters.

D. Adsorption

Virus adsorption is carried out using in series, a wound virus adsorbing filter (No. 4) (or optionally, two of these in series), followed by the 293 plate filter. Insert the wound filter (No. 4) into the virus adsorbing filter holder and place two pieces of media (1.0 and 0.45 micron porosity) into the 293 mm plate filter holder. Be certain that the more open filter (1.0 micron) in on top (upstream) of the finer one (0.45 micron) and that both are in place with their rough side upstream (top) in the water flow.

When the water is diverted to the adsorbing filters, open the bleed valve momentarily to permit the water to fill the holders completely and pass the clarified, chemically adjusted water through the filters, discarding the filtrate. After the adsorption step is complete, disconnect the adsorbing filter holders from the water stream, after redirecting the water stream to drain, and connect the compressed air line and blow all of the residual water from both the wound virus adsorbing filter holder and the plate filter holder.

E. Rinse

After adsorption of the viruses, the adsorbing filters should be rinsed with saline at pH 3.5. This is done to remove residual chemicals which might otherwise find their way into the eluate and adversely effect reconcentration.

Connect in series, the compressor, the large eluate container, the wound virus adsorbing filter holder and the 293 mm plate filter holder. Place approximately 1000 ml of pH 3.5 saline in the eluate container, close its bleed valve, invert it and activate the compressor. As the saline solutions fills the adsorbing filter holders, open the bleed valve on each to allow them to fill completely, closing the valve as soon as liquid is discharged. When the saline has been forced from the eluate container, return it to its normal position and allow the compressor to continue to blow most of the residual liquid from the system. Shut off the compressor and carefully open the valve on the eluate container. Refill the eluate container with another aliquot of pH 3.5 saline and repeat the rinse procedure.

F. Elution

After the saline rinse, place 1000 ml of pH 11.5, 0.1 M glycine into the eluate container, close the bleed valve and invert the container. Actuate the compressor, forcing the alkaline glycine through the adsorbing filters. Open the bleed valves allowing each holder to fill completely. Continue forcing the glycine through the adsorbing filters keeping the total contact time to a minute or two.

Catch the eluate as it discharges from the plate filter holder into 1000 ml of pH 2.0, 1M glycine. When the system is freed of residual liquid return the eluate container to its normal position. Carefully open the bleed valve on the eluate container and place another 1000 ml aliquot of pH 11.5 glycine into it. Repeat the procedure with one change. Catch a small sample of the last effluent from the plate filter and check its pH. It should be between 11 and 11.5. If it is not, virus elution has probably not been complete, and another aliquot of alkaline glycine should be used. This results in fairly large volumes of first eluate, but unless the elution is done completely, virus yields will be correspondingly reduced.

Neutralize the collected eluate by adding pH 2 glycine. This can be done quite quickly without significant loss of viruses. Were the neutralizations to be done by adding 0.1 N HCl, the addition would have to be done slowly with much stirring to minimize virus inactivation. These neutralized glycine eluates can be stored for short periods at refrigerator temperatures without serious loss of virus.

G. Reconcentration

Check pH of first eluate and adjust to pH 7.0 using the necessary glycine solutions. Measure volume of eluate, and while stirring, add enough $AlCl_3$ stock solution to achieve a final concentration of 0.003 M Al^{+++}. (1.0 ml 0.3 M $AlCl_3$ per 100 ml eluate.)

Continue stirring, and add sufficient 1 M Na_2CO_3 dropwise to raise pH to 7.2. Note that the pH will reach 7.2 and tend to drop slowly. Keep adding the Na_2CO_3 until the solution stabilizes at 7.2 for a few minutes.

Stop stirring and let floc settle for 30 minutes. If floc settles well, decant supernate, and centrifuge remaining floc at maximum rpm for 10 minutes. Pour off supernate and discard. Should the flox not settle well, the entire eluate will have to be centrifuted. Pool the pellets from the centrifuge tubes and add sufficient 0.1 M glycine at pH 11.5 to bring well mixed mixture to pH 9.5.

Centrifuge at maximum rpm for 10 minutes. Recover supernate, neutralize to pH 7.5 using pH 2.0 1M glycine concentrate. Add 1.0 ml heat inactivated fetal calf serum per 10 ml concentrate and store in freezer until assay.

IV. OPTIONS AND ALTERNATIVE PROCEDURES

A. Elution of Clarifying Filters

If any of the clarifying filters appear discolored, it is a good idea to elute them also, yielding a concentrate that can be combined with III, I or assayed separately. If assayed separately, it can be determined if there are any virus particles adsorbed on the particulate matter trapped by filters. The filters can be eluted in series with an aliquot of pH 11.5 glycine and immediately neutralized as before (III,F). The first eluate from each of the three clarifying filters can then be combined and reconcentrated as a single sample. Follow the same procedure for reconcentrating as for the adsorbing filter eluates.

B. Batch Method

The batch method is accomplished by filling an appropriate container with clarified sample water, adding sufficient $Na_2S_2O_3$ to yield a negative OTolidine test, adding $AlCl_3$ $6H_2O$ to yield a concentration of 0.0005M (48.3g/100gal), and adding 1MHCl slowly with constant stirring until a pH of 3.5 is obtained.

This clarified, chemically adjusted sample can then be pumped through the water meter and adsorbing filters, in series, bypassing the clarifying section and the proportioning pump. It is necessary to use clarified water for this procedure as the clarifying filters may adsorb a significant amount of virus after the pH is adjusted, and the $AlCl_3$ is added. Sections IIE1, IIE2, IIF1, and IIF2 are omitted if this method is used. Continue at section IIF3 (Alkaline glycine).

1. Receipt and Logging

Virus concentrates as shipped to our laboratories at UNH are delivered from Logan International Airport in Boston by C & J Limo Service, whose drivers are instructed specifically to call at the Airborne Office when virus shipments have been made. The information is communicated at the time of shipment directly to the Carborundum offices and relayed to the staff at UNH. Thus we know when a shipment is expected and can expedite its transfer to UNH.

The coded samples are immediately logged in at the UNH laboratory and assigned a chronological number for record keeping purposes. The condition of the sample at the time of receipt is recorded.

The sample history sheets together with the notations from the UNH personnel are immediately forwarded to our central files at the Carborundum R & D laboratory in Niagara Falls.

2. Storage

Unless the virus concentrates are to be examined immediately, they are placed in a Revco low temperature freezer and maintained at -70°C or lower until they are to be studied.

3. Thawing

At the time they are to be used, samples are removed from the freezer and placed immediately into a water bath at 37°C to thaw.

4. Ether/Antibiotic Treatment

Since membrane filtration to remove bacteria, fungi, etc., has been shown to remove viruses, especially in natural water virus concentrates containing appreciable turbidity, this filtration procedure has not been used. Virus concentrates were treated with 10-15% of their volume of diethylether (Anesthetic Grade) for approximately 16 hours. Enteroviruses are not inactivated by this treatment while bacteria, yeasts and fungi are.

After treatment in a closed tube, residual ether was allowed to evaporate by placing the sample in a tightly covered Petri dish in a laminar flow hood.

More recently (Spring 1977) we found that ether treatment could be eliminated by the use of antibiotics in the concentrates themselves. Our tests showed that even in the case of concentrates obtained from large volumes (50 gal) of unchlorinated sewage effluent, interference during tissue culture by non-viral microorganisms could be prevented by the use of additions of Gentamycin (0.05 mg/ml).

After addition of antibiotics the concentrates are stored for 2 hours at ambient temperature prior to their inoculation onto tissue culture. This storage permits complete inactivation of interfering microorganisms.

5. Hydroextraction

Many virus concentrates arrive from the field sampling in volumes too large for practical tissue culture study. Such samples are further reconcentrated by placing them into a diaysis membrane (Union Carbide Company) which is covered with PEG (polyethylene glycol) (m. wt. 20,000, Grade 20M, Union Carbide) and placed in a refrigerator at 4°C.

After sufficient water has been removed (to approximately 5-10 ml) the concentrates are recovered by rinsing the dialysis tubing with 5-15 ml of 10% beef extract raised to pH 9 by the addition of PBS buffer. Typical volumes after hydroextraction range from 10 to 50 ml. This material is innoculated in its entirety onto suitable tissue.

6. Virus Assay

A. Tissue Cultures

Two types of tissue cultures are used. A continuous cell line, Buffalo Green Monkey (BGM) is maintained by passage of a BGM culture originally obtained from Dr. R. Engelbrecht (University of Illinois), who originally obtained it from Dr. G. Berg, (EPA, Cincinnati). This BGM cell line was originally derived from African Green Monkey kidney cells.

Periodically, each lot of BGM cells is used to titer a control stock of virus, which was produced from a low passage of a natural virus isolant. This procedure assures that during repeated passages, this continuous BGM cell line has not suffered decreased sensitivity. Should such a decrease be noted, a low passage number of stock BGM cells is removed from the freezer and substituted.

The second cell line generally used is grown from Primary Monkey Kidney Cells (PMK) of African Green Monkey origin. These cell suspensions are received on a regular basis from Microbiological Associates.

B. Preparation of Cell Sheets

To produce the tissue cultures, suspensions of dispersed cells are inoculated into 6 oz. prescription bottles at the rate of 3 x 10^6 cells per bottle for BGM, and 5 x 10^6 cells per bottle for PMK. Growth media consist of MEM-L15 with 10% FCS for BGM cells and Melnicks A with 2% FCS for PMK cells.

Rubber stopped bottles are incubated at 37°C for 3-4 days with BGM, 7 days with PMK. Browth media is changed when required. This is shown by a shift in the pH indicator from pink to yellow, brought about by the formation of acidic metabolism by-products.

Cell sheets reach confluency and are used, typically in 5 to 7 days.

C. Inoculate

Cell sheets are carefully drained prior to inoculation.

To each cell sheet (6 oz. bottle) is added a 0.5 ml inoculum. The bottle is carefully rotated and rocked to assure uniform distribution of the inoculum, after which viruses are allowed to adsorb for 1 to 1½ hours under static conditions at room temperature. After adsorption, the inocula are drained off and the cell sheets are rinsed. Each rinse utilizes 2 ml of PBS. The number of such rinses varies depending on the appearance of the concentrate. As many as five rinses may be used.

Our studies with various exogenously seeded samples have shown that few if any viruses are lost by such rinsing procedures.

Each concentrate is plaqued using two different cell lines BGM and PMK to improve the chance for virus recovery. In addition, only half of a concentrate is examined at one time, onequarter on each type of cell culture. This is done primarily to safeguard against the loss of an entire sample should problems be encountered during the tests. Such problems as cytotoxicity, contamination, equipment failure, etc., have rarely been encountered, however. Plaquing was selected over liquid overlay methods in order that a direct count of virus isolants could be obtained. In addition, plaquing permits the individual isolants to be picked directly for identification without the need of an additional plaque purification step. It should be remembered that plaquing is nota "cure all" since virus demonstration by plaquing is generally less sensitive than by CPE, even with those viruses which plaque. In addition, a number of viruses plaque only with great difficulty, and some not at all.

After adsorbing the virus to the tissue culture and rinsing to minimize the possibility of cytotoxic effects, the cell sheets are overlaid with suitable agar overlay media. The bottles are placed on the bench, and covered to prevent light interaction with the vital dye and possible adverse effects on the tissue cultures. After the gel has solidified, the bottles are inverted and incubated at 37°C. They are examined repeatedly until the quality of the cell sheets starts to degrade excessively. Plaques are counted and marked each time the bottles are examined. In a large portion of the cases, cell quality remains acceptably good for periods of 2 to 3 weeks. Such prolonged incubation results in the recovery of many "slow" plaque forming viruses. Natural virus isolants are characteristically slower to develop plaques than are laboratory strains of the same viruses.

Along with each set of tissue cultures, several bottles are maintained as controls, receiving identical treatment as those receiving virus concentrate except for the inoculation itself. To date, no control has developed plaques or cytotaxicity, proving that the quality of the laboratory safeguards is excellent.

7. Virus Confirmation

Each observed plaque, at this point considered a tentative virus isolant, is confirmed by making repeated (usually 4) picks around its periphery using a curved Pasteur pipet. The picks are transferred to a cell culture of the same type in order to eliminate possible effects of cell specificity.

To each bottle is added an overlay medium consisting of Melnick's B, the composition used throughout our laboratory for the maintance of tissue cultures. They are incubated at 37°C and examined daily for the development of cytopathic effects (CPE). This provides confirmation of the viral etiology of the observed plaque.

At this point, it should be mentioned that many investigators are reporting that a large proportion, even in some cases more than half, of their observed plaques do not pass and produce CPE (personal communications, and presentations ASM Meeting, Las Vegas, May 1978). For whatever reason, we have not had this trouble. Only rarely will a plaque fail to produce CPE on passage. Several of those which failed to produce CPE were examined in some detail. We feel that our tests prove that the plaques were viral, and that toxicity, contamination, etc., can be ruled out as probable cause. We prefer, therefore, to list them as plaques, and state simply that they failed to yield CPE on picking and passing.

8. Virus Identification

After the passed virus had produced extensive CPE in the cell culture under liquid overlay, the contents of the bottle are quickly frozen and thawed three times to lyse the cells and free progency virus. The liquid is centrifuged at low speed to remove cellular debris and the resulting virus preparation is identified by serum neutralization.

Serial dilutions of each such preparation are titrated to determine the virus concentration. Aliquots of the virus suspension (0.025 ml containing 50 - 500 pfu) are mixed with appropriate antisera (0.025 ml containing 10 units), reacted for 1 hour at room temperature than transferred to the microtiter plates. After 1 hour adsorption at 37°C. As stated before, each experiment is run with suitable controls, both positive and negative as required.

In order to conserve the limited supply of Lim-Benyesh-Melnick (LBM) antiserum pools, we established a some what different procedure for serum neutralization tests.

The majority of natural virus isolants have been shown to be one of the polioviruses. Therefore, we prepared a pool of types 1, 2 and 3 polio antisera. (Microbiological Associates.) Each passaged isolant is first screened against these pools. This, although increasing the time required for final confirmation of non-polio isolants and mixtures greatly reduces the amount of LBM antiserum pool used.

Those viruses not yielding a clear identification with the mixed polio antiserum are tested against the LBM pool. Finally, those not clearly identified are tested against homotypic Coxsackie B-3 antiserum. This particular virus is not identified by the LBM pool, and has been, in fact, one of our more common isolants from Lexington, Missouri waters. Should none of these techniques yield a definite identifiable pattern of serum neutralization, the plaque is listed as unidentified, or a mixture, depending on the specifics of the neutralization tests.

The remainder of each passaged isolant is placed in a Revco freezer and stored at -70°C or lower for possible future studies.

9. Reporting

Each sample from the time it was taken, is accompanied by suitable data sheets to permit us to know clearly its present status and what was done at each step of the way. The sample was coded at the time it was taken with the contract designation (UZ), (year-month-day-780124), the operator's initials (B.M.), and other designations as necessary. A sheet with pertinent information accompanies each sample to the virus laboratory.

At the virus laboratory, the date, time and condition of each sample as received is noted, with the observers initials, and the sample is assigned a chronological laboratory number. This number is noted on the sample sheet which is forwarded to our central data files in Niagara Falls.

As the analysis proceeds, data are periodically transmitted by telephone and such tentative results are included in various reports. Upon completion of the analysis and isolant identification, a final data sheet is forwarded to Niagara Falls. All sample information, shipments, analysis and reports are checked, any discrepancies resolved, and the results are then reported as final.

APPENDIX B

SUMMARY OF VIRUS ASSAY RESULTS AND MARKER T TESTS

The "T40°C" marker (T) test was carried out in vitro in poliovirus isolates to determine virulence. The T marker refers to the ability of virulent (T+) isolates to grow as well, or nearly as well, at 40°C as at the normal (37°C) incubation temperature. On the other hand, an attenuated (T-) strain shows a marked reduction in titer at these elevated temperatures.

As an aide in evaluating the T marker values, the Reproductive Capacity Temperature Marker factor (rct/40 test) was determined. The rct/40 test allows comparison of the T+ and T- characteristics of virulent or non-virulent isolates and enables assignment of relative values to known polio 1 types as well as to the unknown isolate. This calculated R value allows classification of a particular isolate in relation to known virulent and non-virulent strains. "R" values are given by:

$$R = \frac{Xr - A_o r}{V_o r - A_o r} \times 100$$

where:

$$r = \frac{\text{plaque forming units after 18 hours at } 40^\circ\text{C}}{\text{plaque forming units after 18 hours at } 37^\circ\text{C}}$$

Xr = r value of an unknown

$A_o r$ = r value of an avirulent control, polio LSc-1

$V_o r$ = r value of the virulent control, mahoney strain of polio I

R values signifying virulent (wind type) poliovirus were obtained for four (4) samples (one 95.7 and three 100 percent). Two R values of 55.6 and 53 percent indicate isolants of a virulent quality. R values for the remaining isolants indicate poliovirus of differing degrees of virulent quality.

Legend

A - Raw water
B - Presettled raw water
C - Softened water
D - Disinfected, treated with chlorine, chloramine, die-off
F - Finished water, all such samples shipped to EPA
P - Pilot Plant
(T) - Sample collected in 500 gallon tank prior to sampling

APPENDIX B-1. SUMMARY OF RAW MISSOURI RIVER ENTEROVIRUS ASSAY DATA AT LEXINGTON, MISSOURI

Date	Virus Ct.	Prefilter								Absorber								OTHER	UNIDENTIFIED
		P1	P2	P3	CB1	CB2	CB3	CB4	CB5	P1	P2	P3	CB1	CB2	CB3	CB4	CB5		
1976																			
12/14	3	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0
12/18	5	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0
1977																			
1/4	2	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
1/14	9	0	0	0	0	0	0	0	0	2	-	1	-	4	-	-	-	-	2(A)
1/17	19	0	0	0	0	0	0	0	0	1	6	1	-	1	-	-	-	-	10(A)
2/3	7	0	0	0	0	0	0	0	0	3	-	1	-	-	-	1	-	-	2(A)
2/4	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
2/7	6	0	0	0	0	0	0	0	0	1	3	-	-	-	-	-	-	-	2(A)
2/10	8	0	0	0	0	0	0	0	0	2	3	-	-	-	-	-	-	-	3(A)
2/14	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
2/17	5	0	0	0	0	0	0	0	0	1	3	-	-	-	-	-	-	-	1(A)
2/22	10	0	0	0	0	0	0	0	0	2	6	-	-	-	-	-	-	-	2(A)
3/3	8	1	1	1	0	0	2	1	0	0	1	1	0	0	0	0	0	0	0
3/7	6	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	2(A),1(C)
3/10	5	0	1	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
3/14	21	1	1	-	-	-	-	-	-	2	4	2	-	2	-	-	-	-	7(A),2(C)
3/17	12	0	1	4	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0
3/21	11	2	1	0	3	0	0	0	0	1	3	1	0	0	0	0	0	0	0
3/24	9	2	2	1	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
3/26	7	0	1	0	0	0	0	0	0	3	1	-	-	1	-	-	-	-	1(A)
4/18	7	0	0	0	0	0	0	0	0	0	4	2	0	1	0	0	0	0	0
4/21	4	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
4/25	5	0	0	0	0	0	0	0	0	3	1	0	0	1	0	0	0	0	0
4/28	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

Notes: Prefilter ≡ Virus recovered from solids (>3 to 5μ) retained by cartridge filter.

(A) = unidentified virus recovered from absorber

(C) = " " " " prefilter

Appendix B-1 (continued)

Date	Virus Ct.	Prefilter								Absorber								OTHER	UNIDENTIFIED
		P1	P2	P3	CB1	CB2	CB3	CB4	CB5	P1	P2	P3	CB1	CB2	CB3	CB4	CB5		
5/2	3	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
5/16	2	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
5/19	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
6/13	10	-	-	-	-	-	-	-	-	0	1	0	0	0	5	3	0	-	1(C)
6/16	3	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0
6/23	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
6/29	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
7/11	4	0	0	0	0	0	0	0	0	-	-	2	-	-	1	-	-	-	1(A)
7/14	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
7/18	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
7/21	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
7/25	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
7/28	2	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
8/3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-1(A)	0
8/16	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(C)
8/29	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-1(C) 2 Echo-7(C)	0
9/1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9/15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
9/18	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
9/19	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
9/21	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9/29	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-7(C)	0
10/3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-7(C)	0
10/6	1	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0	1(C)

Notes: Prefilter ≡ Virus recovered from solids (>3 to 5μ) retained by cartridge filter.

(A) = unidentified virus recovered from absorber

(C) = " " " " " prefilter

APPENDIX B-1 (continued)

Date	Virus Ct.	Prefilter								Absorber								OTHER	UNIDENTIFIED
		P1	P2	P3	CB1	CB2	CB3	CB4	CB5	P1	P2	P3	CB1	CB2	CB3	CB4	CB5		
3/2	9	0	0	0	0	0	0	0	0	1	2	6	0	0	0	0	0	0	0
3/6	10	0	0	0	0	0	0	0	0	2	3	4	-	-	-	-	-	-	1(A)
3/7	5	0	0	0	0	0	0	0	0	-	3	1	-	-	-	-	-	-	1(A)
3/9	7	0	0	0	0	0	0	0	0	0	2	5	0	0	0	0	0	0	0
3/13	5	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0
3/20	8	0	1	0	0	0	0	0	0	0	4	2	-	-	-	-	-	-	1(A)
3/21	2	0	0	0	0	0	0	0	0	-	-	1	-	-	-	-	-	-	1(A)
3/28	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	-	0
3/29	3	0	0	0	0	0	0	0	0	1	-	-	-	-	-	-	-	-	2(A)
4/3	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
5/8	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
9/25	5	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	1 Echo-1(A)	0
10/2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-1(A) 1 Echo-7(A)	0
10/10	7	0	0	0	0	0	0	0	0	0	3	1	0	1	0	0	1	1 Echo-7(A)	0
10/17	4	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2 Echo-7(A)	0
10/23	3	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
10/30	2	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
11/6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1-Echo-7(A)	0
11/27	5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	3 Echo-7(A)	0
12/4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-7(A)	0
12/11	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1 Echo-7(A)	0
12/27	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2 Echo-7(A)	0

Notes: Prefilter ≡ Virus recovered from solids (>3 to 5μ) retained by cartridge filter.

(A) = unidentified virus recovered from absorber

(C) = " " " " prefilter

APPENDIX B-1 (continued)

Date	Virus Ct.	Prefilter								Absorber								OTHER	UNIDENTIFIED
		P1	P2	P3	CB1	CB2	CB3	CB4	CB5	P1	P2	P3	CB1	CB2	CB3	CB4	CB5		
11/7	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11/10	6	0	1	1	0	0	0	0	0	-	-	1	-	-	-	-	1	1 Echo-14(A)	1(A)
11/14	2	-	-	-	-	-	-	-	-	0	1	0	0	0	0	0	0	-	1(C)
11/17	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1 Echo-14(A)	0
11/21	3	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
11/29	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1 Echo-7(A)	0
12/1	6	0	0	0	0	0	0	0	0	0	5	0	0	0	0	1	0	0	0
12/5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-7(A)	0
12/8	4	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	0	0	0
12/12	10	0	0	0	0	0	0	0	0	1	4	2	-	-	-	-	-	2 Echo-7(A)	1(A)
12/14	2	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
12/22	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
12/27	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
1978																			
1/3	1	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	1(A)
1/4	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1/5	2	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
1/9	4	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	4(A)
1/11	14	0	0	0	0	0	0	0	0	-	7	4	-	-	-	-	-	1 Echo-7(A)	2(A)
1/26	3	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
1/30	5	0	0	0	0	0	0	0	0	-	1	3	0	0	0	0	0	-	1(A)
2/2	3	0	0	0	0	0	0	0	0	2	-	-	-	-	-	-	-	-	1(A)
2/7	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
2/9	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
2/14	5	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0	0	0
2/16	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
2/22	8	0	0	0	0	0	0	0	0	-	5	2	-	-	-	-	-	0	1(A)
2/23	5	0	0	0	0	0	0	0	0	0	2	2	0	0	1	0	0	0	0
2/27	8	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	0	0	0

Notes: Prefilter ≡ Virus recovered from solids (>3 to 5μ) retained by cartridge filter.

(A) = unidentified virus recovered from absorber
(C) = " " " " prefilter

APPENDIX B-2. SUMMARY OF ENTEROVIRUS ASSAY DATA FOR PRESETTLED AND LIME SOFTENED MISSOURI RIVER WATER AT LEXINGTON, MISSOURI

Date	Virus Ct.	Prefilter								Absorber								OTHER	UNIDENTIFIED
		P1	P2	P3	CB1	CB2	CB3	CB4	CB5	P1	P2	P3	CB1	CB2	CB3	CB4	CB5		
1976																			
12/20	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1977																			
1/11	8	0	0	0	0	0	0	0	0	2	2	-	-	1	-	-	-	-	3(A)
1/18	6	0	0	0	0	0	0	0	0	1	3	0	0	1	1	0	0	0	0
8/22	1	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	1(A)
10/20	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 Echo-7(A)	0
10/26a	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1978																			
3/9	6	0	0	0	0	0	0	0	0	1	4	1	0	0	0	0	0	0	0
3/13	5	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0
3/22	10	0	0	0	0	0	0	0	0	3	3	-	-	-	-	-	-	-	4(A)
1977	(Lime Softened Mo. River Water)																		
1/12	5	0	0	1	0	0	0	0	0	0	2	0	0	1	0	1	0	0	0
1/20	2	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0

Notes: Prefilter ≡ Virus recovered from solids (>3 to 5μ) retained by cartridge filter.

(A) = unidentified virus recovered from absorber

(C) = " " " " prefilter

APPENDIX B-3 RESULTS OF MARKER (T) TESTS FOR POLIOVIRUS ISOLANTS RECOVERED FROM LEXINGTON, MISSOURI

Date of Collection	Site	Lab #	Isolate #	Temp°C	Titer (pfu/ml)	r Value	R Value
12/18/76	A	1019	4	40 37	$1.9x^{6}$ $3.4x10^{6}$	.558	55.6
2/17/77	A(T)	1182	3	40 37	$2.95x10^{6}$ $3.25x10^{7}$	.0907	1
1/19/77	B	1107	-	40 37	$4.85x10^{6}$ $5.15x10^{7}$	.094	1.3
1/14/77	A	1090	2	40 37	$1.75x10^{6}$ $2.85x10^{7}$	.061	1
Controls for samples listed above:							
LSC-1				40 37	$3.1x10^{6}$ $3.75x10^{7}$	.083	
3/13/78 B	B	2024	1	40 37	$4.05x10^{5}$ $9.2x10^{6}$	.044	5.9
2/09/78 B	D	1996	1	40 37	dng* $2.6x10^{6}$	0	1
3/06/78	A	2014	1	40 37	$5.7x10^{5}$ $1.4x10^{7}$		
9/19/77	A(T)	1802	1	40 37	$4.75x10^{6}$ $2.3x10^{7}$	.2065	27.8
3/14/77	A(T)	1220	3	40 37	$8.8x10^{4}$ $3.1x10^{7}$	.00285	1
3/02/78 B	P	2011	1	40 37	$9.7x10^{4}$ $2.6x10^{7}$	.00375	1
2/09/78 A	A(T)	1995	1	40 37	$5.3x10^{3}$ $3.15x10^{6}$	.00168	1
Controls for Samples listed above:							
LSC-1				40 37	dng* $3.9x10^{6}$	0	
Mahoney				40 37	$4.6x10^{6}$ $6.2x10^{6}$	.742	

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*dng - means did not grow

APPENDIX B-3 (continued)

Date of Collection	Site	Lab #	Isolate #	Temp°C	Titer (pfu/ml)	r Value	R Value
12/18/76	A	1019	1	40	$8.5x10^{5}$	.0548	1
				37	$1.55x10^{7}$		
1/17/77	A	1105	1	40	$4.1x10^{5}$	.15	3.2
				37	$2.75x10^{6}$		
3/03/77	A(T)	1206	2	40	$6.8x10^{5}$	.042	1
				37	$1.6x10^{7}$		
11/21/77	A(T)	1926	1	40	$3.5x10^{6}$	.041	1
				37	$8.5x10^{7}$		
3/10/77	A(T)	1218	1	40	$4.6x10^{6}$	.092	1
				37	$5.0x10^{7}$		
12/14/77	A(T)	1955	1	40	$2.45x10^{7}$	.79	95.7
				37	$3.1x10^{7}$		
3/07/78 B	D	2016	-	40	$5.8x10^{7}$	.374	35.5
				37	$1.55x10^{8}$		
Controls for samples listed above:							
LSC-1				40	$2.5x10^{6}$	.128	
				37	$1.9x10^{7}$		
Mahoney				40	$2.5x10^{7}$	.820	
				37	$3.0x10^{7}$		
4/25/77	A(T)	1390	5	40	$9.05x10^{5}$	.0139	1.9
				37	$6.5x10^{7}$		
4/25/77	A(T)	1390	-	40	$6.8x10^{5}$	.0129	1.8
				37	$5.25x10^{7}$		
3/21/77	A(T)	1273	1	40	$3.6x10^{4}$	.0005	1
				37	$6.55x10^{7}$		
12/12/77	A(T)	1953	2	40	$1.5x10^{3}$	.00002	1
				37	$7.25x10^{7}$		
4/25/77	A(T)	1390	1	40	$7.2x10^{5}$	.016	2.3
				37	$4.5x10^{7}$		
1/14/77	A	1090	3	40	$1.85x10^{4}$	.0025	1
				37	$7.25x10^{6}$		
3/14/77	A(T)	1220	4	40	$2.3x10^{6}$	.046	6.7
				37	$4.95x10^{7}$		
Controls for samples listed above:							
LSC-1				40	$6.0x10^{2}$	.0002	
				37	$2.25x10^{6}$		

APPENDIX B-3 (continued)

Date of Collection	Site	Lab #	Isolate #	Temp$^{\circ}$C	Titer (pfu/ml)	r Value	R Value
Mahoney				40	$2.2x10^6$	.688	
				37	$3.2x10^6$		
11/17/77 A	A(T)	1922	1	40	$4.5x10^7$	1.08	100
				37	$4.15x10^7$		
3/02/78 A	A(T)	2010	7	40	$1.0x10^2$	.00005	1
				37	$2.05x10^7$		
2/22/77	A(T)	1201	2	40	dng*	0	1
				37	$1.8x10^7$		
2/09/78 B	D	1996	2	40	$4.7x10^5$	.023	2.4
				37	$2.0x10^7$		
2/02/78	A(T)	1991	3	40	$2.3x10^7$	1.05	100
				37	$2.2x10^7$		
12/22/77	A(T)	1959	1	40	$2.8x10^6$	1.24	100
				37	$2.25x10^6$		
3/06/78	A	2014	2	40	dng*	0	1
				37	$9.0x10^6$		
2/22/77	A(T)	1201	4	40	dng*	0	1
				37	$5.7x10^6$		
Controls for samples listed above:							
LSC-1				40	dng*	0	
				37	$1.35x10^7$		
Mahoney				40	$2.65x10^7$	.946	
					$2.8x10^7$		
3/08/78 B	P	2017	1	40	$2.65x10^5$	.16	25.0
				37	$1.65x10^6$		
3/24/77	A(T)	1277	2	40	$1.75x10^7$	.327	53.0
				37	$5.35x10^7$		
7/25/77	A(T)	1661	1	40	$3.25x10^6$	.023	1.8
				37	$1.4x10^8$		
Controls for samples listed above:							
LSC-1				40	$2.6x10^5$	.012	
				37	$2.25x10^7$		
Mahoney				40	$1.9x10^7$	.603	
				37	$3.15x10^7$		

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*dng - did not grow

APPENDIX C-1 SUMMARY OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL DATA CORRESPONDING TO RAW MISSOURI RIVER VIRUS ASSAY DATA AT LEXINGTON, MISSOURI

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
1976										
12/3	0	28,400	6,000	-----	----	174	248	70*	0.0	38,100
12/6	0	198,000	5,800	14,000	----	162	136	85*	0.0	44,500
12/14	6	21,300	9,300	-----	----	188	266	43*	0.5	26,600
12/18	10	314,000	20,200	500	----	180	260	50*	3.0	29,100
1977										
1/4	4	84,000	0	-----	----	198	268	33*	0.0	23,600
1/14	18	470,000	24,000	18,000	55	226	288	3.5	0.0	20,700
1/17	38	17,000	1,900	-----	15	218	290	5.0	0.0	15,200
1/18	0	-----	-----	-----	----	214	292	3.1	0.0	17,400
2/3	4	18,000	-----	5,000	0	192	260	3.9	0.0	17,200
2/4	14	3,500	650	12,500	0	196	264	3.9	0.0	15,400
2/7	6	24,000	1,100	5,000	30	212	272	4.8	0.0	16,400
2/8	0	-----	-----	-----	----	214	268	---	0.5	19,100
2/10	8	55,000	8,500	11,000	40	208	247	3.9	2.0	23,300
2/11	0	-----	-----	-----	----	214	280	---	2.0	24,800
2/14	2	100,000	5,000	8,000	45	188	250	6.6	1.5	27,900
2/15	0	-----	-----	-----	----	182	240	---	1.5	30,000
2/17	5	33,000	6,000	4,000	100	176	236	5.3	1.0	30,400
2/18	0	-----	-----	-----	----	174	230	---	1.0	33,800
2/22	10	21,000	85	6,000	135	172	230	9.9	1.5	30,300
2/23	0	-----	-----	-----	----	162	226	---	2.0	51,700
2/28	0	2,000	0	350	40	166	208	46	3.0	33,000

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-1 (continued)

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
3/1	0	-----	-----	-----	----	160	210	---	4.0	31,300
3/3	8	90,000	11,700	17,000	150	160	202	49	4.0	29,700
3/4	0	-----	-----	-----	----	168	218	---	3.5	29,100
3/7	6	34,000	6,700	5,000	60	176	220	41	4.0	27,100
3/8	0	-----	-----	-----	----	172	228	---	5.0	27,600
3/10	5	25,000	7,900	5,400	80	176	220	22	7.5	27,600
3/14	21	8,500	4,400	15,600	100	174	212	38	9.0	25,500
3/15	0	-----	-----	-----	----	166	216	34	9.5	31,400
3/17	12	56,000	9,300	14,000	28	166	212	44	9.0	34,600
3/18	0	-----	-----	-----	----	164	218	43	9.0	32,000
3/21	11.7	17,000	2,100	24,000	16	160	216	67	7.0	33,600
3/22	0	-----	-----	-----	----	162	---	53	6.0	33,000
3/24	17.3	5,600	8,800	24,000	40	158	210	70	7.0	42,700
3/25	0	-----	-----	-----	----	148	210	---	7.5	40,800
3/26	11	-----	-----	-----	----	130	212	63	8.5	38,400
3/28	0	3,200	7,700	15,600	36	148	200	66	10.5	43,300
3/29	0	-----	-----	-----	----	140	214	39	10.5	41,900
4/1	0	920,000	11,000	-----	13	156	206	80	9.0	48,000
4/2	0	-----	-----	-----	----	144	210	62	9.0	45,700
4/4	0	160,000	8,900	3,500	15	160	216	55	8.0	45,800
4/5	0	-----	-----	-----	----	158	222	44	7.0	48,000
4/13	0	-----	-----	-----	----	152	226	44	14.5	40,900
4/14	0	11,400	3,000	2,100	5	164	172	44	15.5	40,900
4/18	7	21,000	8,500	5,000	9	160	126	32	16.0	40,300
4/19	0	-----	-----	-----	----	154	230	27	16.5	42,000
4/21	8	590,000	80,000	72,000	130	148	218	83	17.0	42,100
4/25	5	400,000	7,300	9,200	28	164	214	61	14.5	47,400
4/26	0	-----	-----	-----	----	164	224	57	15.5	44,600
4/28	1	70,000	12,000	16,000	8	158	212	53	16.5	42,100
4/29	0	-----	-----	-----	----	160	220	39	16.5	42,200

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-I (continued)

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. ^oC	River Flow CFS
5/2	3	54,000	2,400	17,500	23	162	162	39	17.5	40,300
5/5	0	2,910,000	10,500	21,300	150	154	200	205	19.0	42,300
5/9	0	750,000	210	-----	175	152	210	200	19.5	45,700
5/12	0	578,000	23,500	15,000	111	152	212	210	19.5	42,700
5/16	2.9	405	9,000	19,000	18	154	222	130	21.0	39,000
5/19	1.9	1,750,000	210	38,500	22	160	220	64	22.5	40,100
5/20	0	-----	-----	-----	----	142	218	64	19.5	57,100
5/23	0	81,300	13,900	-----	123	136	184	225	21.0	55,000
5/26	0	158,000	2,100	71,500	32	144	186	230	23.0	56,600
5/27	0	-----	-----	-----	----	132	184	230	23.5	52,000
6/1	0	865,000	37,000	112,000	217	138	184	200	23.5	64,200
6/6	0	98,500	6,100	33,000	32	144	182	180	24.0	54,000
6/9	0	139,000	10,000	43,500	11	148	190	150	24.0	45,700
6/13	10	300,000	7,900	243,000	129	156	208	75	24.0	43,500
6/16	6	131,000	6,500	43,000	11	156	214	54	24.5	46,200
6/20	0	210,000	20,000	291,000	150	126	126	200	24.5	99,700
6/21	0	-----	-----	-----	----	118	164	220	24.0	88,500
6/23	4	154,000	14,400	121,000	61	112	146	205	24.0	89,400
6/24	0	-----	-----	-----	----	110	144	215	24.0	78,500
6/27	0	137,000	8,400	114,000	62	134	174	195	25.5	79,100
6/28	0	-----	-----	-----	----	128	174	205	26.5	70,400
6/29	2	-----	-----	-----	----	124	172	---	26.5	67,000
6/30	0	177,000	10,100	204,000	161	130	174	---	26.0	65,400

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-1 (continued)

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
7/6	0	-----	-----	-----	----	140	210	245	27.0	45,700
7/7	0	282,000	10,500	35,000	20	146	210	---	27.0	46,800
7/8	0	102,000	0	18,500	8	136	204	---	27.0	48,100
7/11	8	122,000	24,000	52,500	23	150	216	85	26.0	48,700
7/12	0	-----	-----	-----	----	134	204	---	27.0	49,600
7/14	2	150,000	21,200	67,000	8	148	214	115	27.0	44,400
7/15	0	-----	-----	-----	----	142	222	---	27.5	44,800
7/18	1.6	11,500	68,500	13,200	11	156	198	45	28.0	44,000
7/21	1.5	77,000	4,450	450,000	21	158	230	40	28.0	43,000
7/25	1.3	64,200	6,500	61,000	29	154	250	80	28.0	44,400
7/28	2.2	84,000	5,400	18,500	7	152	214	80	26.5	42,600
8/1	0	103,000	8,000	-----	27	144	230	130	26.5	38,800
8/3	1.1	530,000	3,700	38,000	29	134	190	---	27.0	39,200
8/8	0	253,000	8,000	82,000	306	116	180	220	27.0	54,800
8/16	1.6	134,000	10,000	185,000	59	128	210	190	24.5	50,500
8/22	1.9	108,000	17,500	-----	16	124	208	205	25.0	49,000
8/29	4.2	190,000	0	57,500	132	134	200	125	23.0	85,900
9/1	3.3	378,000	39,000	195,000	710	110	158	200	22.5	108,000
9/13	0	435,000	46,000	810,000	----	80	120	160	21.0	200,000
9/14	0	234,000	21,000	720,000	1,350	82	120	220	20.5	186,000
9/15	2	155,000	18,000	86,000	695	86	116	230	21.0	131,000
9/17	0	205,000	37,000	59,000	550	98	134	225	22.0	97,200
9/18	4	-----	-----	-----	----	128	156	---	21.0	86,300
9/19	2	184,000	6,300	37,100	110	126	180	130	22.0	86,100
9/20	0	82,000	4,600	106,000	42	132	180	123	22.0	81,500
9/21	2	106,000	11,200	320,000	180	136	184	170	21.0	77,200
9/22	0	130,000	17,000	980,000	143	130	182	160	20.5	74,100
9/26	0	91,300	10,000	120,000	900	130	190	200	19.5	76,300
9/29	2	170,000	13,300	122,000	240	140	214	120	19.5	59,500

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-1 (continued)

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
10/3	2	90,500	2,500	6,100	28	158	214	63	18.0	55,200
10/6	2	335,000	9,000	15,000	128	160	230	70	15.0	50,300
10/10	0	154,000	12,500	58,000	2,680	136	194	160	13.0	73,900
10/13	0	190,000	8,800	90,500	208	158	214	84	11.0	53,900
10/17	0	155,000	39,000	14,700	41	168	234	41	11.5	50,200
11/7	2	87,000	5,900	3,000	810	154	216	90	13.0	66,000
11/8	0	124,000	4,700	46,600	77	162	216	62	13.0	59,900
11/10	10	970,000	25,000	-----	755	136	190	200	9.0	154,000
11/14	3.7	204,000	53,000	60,000	1,040	150	208	80	8.0	73,800
11/17	2.9	415,000	14,700	36,000	119	170	225	92	7.5	65,300
11/21	3	200,000	9,800	14,600	84	172	246	40	6.0	62,300
11/29	4.1	132,000	6,600	19,500	47	186	268	40	1.0	42,500
12/1	8.8	173,000	18,500	33,000	30	194	274	34	2.0	38,600
12/5	1	230,000	150,000	27,000	23	210	270	28	0.5	35,700
12/8	4.4	360,000	-----	41,000	35	218	284	34	0.5	27,100
12/12	10	192,000	9,900	17,500	29	250	316	27	1.0	19,400
12/13	0	-----	-----	-----	----	250	316	---	1.0	25,500
12/14	2	-----	-----	-----	----	244	320	27	2.0	28,500
12/15	0	280,000	11,800	17,500	40	248	320	30	2.0	28,200
12/19	0	153,000	8,300	16,200	27	204	274	30	3.0	36,600
12/20	0	-----	-----	-----	----	202	268	26	1.5	38,300
12/22	1	375,000	16,500	30,600	225	196	270	27	0.0	33,600
12/27	2	184,000	9,200	20,400	50	206	278	20	1.0	29,600
12/28	0	-----	-----	-----	----	208	278	16	1.0	29,100

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-I (continued)

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
1978										
1/3	1	76,500	5,700	41,000	28	212	288	20	0.0	28,900
1/4	1	505,000	10,500	21,600	66	208	282	16	0.5	27,900
1/5	2	475,000	8,900	7,200	66	208	280	16	1.0	27,800
1/9	4	340,000	9,800	24,200	20	216	298	17	0.0	29,200
1/11	14	28,000	3,400	6,700	17	214	286	12	0.0	24,700
1/19	0	172,000	6,800	36,800	36	220	296	7.0	0.0	17,600
1/23	0	167,000	11,600	27,000	7	224	298	8.6	0.0	------
1/26	3	125,000	8,400	4,100	7	216	292	11	0.0	21,400
1/30	5	38,500	3,800	3,300	7	210	294	8.0	0.0	18,200
2/2	3	152,000	6,200	8,500	0	228	286	2.5	0.0	19,400
2/7	1	84,000	2,300	17,600	0	222	280	3.0	0.0	21,000
2/9	1	77,000	4,400	12,800	2	212	288	2.8	0.0	21,000
2/14	5	84,000	6,300	8,200	3	208	278	2.8	0.0	24,000
2/16	2	90,000	12,800	6,800	3	194	266	3.1	0.0	25,000
2/22	8	54,800	3,400	4,500	6	204	278	2.2	1.0	24,000
2/23	5	36,000	3,000	2,100	0	202	274	2.9	1.5	25,000
2/27	8	20,500	1,200	6,300	8	96	188	0.05	1.5	32,700
3/2	11.7	193,000	10,400	27,500	27	182	258	40	1.5	25,900
3/6	10	128,000	4,500	31,500	15	174	250	22	1.0	26,000
3/7	5.3	-----	-----	-----	----	180	250	19	1.0	27,500
3/9	7	63,300	2,800	15,800	32	186	274	19	2.5	31,500
3/13	6.8	112,000	3,600	56,500	33	144	190	110	3.5	62,900
3/14	1.7	184,000	4,400	81,000	76	136	190	130	1.5	74,200
3/20	8	15,300	100	35,000	24	110	140	105	2.0	172,000
3/21	2.8	-----	-----	-----	----	110	122	53	2.5	175,000
3/28	1	82,800	7,100	95,000	50	130	158	48	6.5	113,000
3/29	1	-----	-----	-----	----	136	178	33	7.5	98,200
		150,000	5,100	42,000	81	134	174	51	9.0	87,000

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-1 (continued)

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
4/3	1	133,000	5,600	9,800	41	122	170	---	13.0	76,300
4/13	0	132,000	11,000	86,000	455	144	154	180	13.0	98,300
4/17	0	106,000	3,600	43,500	185	154	212	180	10.0	82,200
4/24	0	155,000	10,000	99,000	79	154	196	130	9.5	101,000
5/1	0	62,000	3,600	47,500	55	174	224	130	12.0	69,000
5/8	1	93,500	14,400	111,000	126	178	226	---	11.0	145,000
5/15	0	80,500	7,500	59,500	57	164	204	170	16.0	80,400
5/22	0	98,000	5,000	42,000	19	176	238	170	19.0	70,100
5/30	0	39,000	5,000	40,700	48	170	224	180	21.5	71,500
6/5	0	44,000	4,400	20,800	23	140	190	140	20.5	76,500
6/19	0	-----	-----	-----	----	172	232	170	25.5	53,400
7/3	0	48,700	3,500	56,000	8	162	220	105	28.0	57,000
7/10	0	102,200	7,400	142,000	76	146	---	160	25.0	72,100
7/17	0	49,700	650	85,000	114	146	---	220	26.0	62,200
7/24	0	129,000	26,000	369,000	735	108	---	---	24.5	127,000
7/31	0	88,000	5,200	70,000	21	140	214	100	26.0	76,200

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-1 (continued)

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
8/7	0	-----	-----	-----	----	158	230	300*	24.0	70,300
8/14	0	-----	-----	-----	----	168	232	350*	27.0	61,900
8/21	0	-----	-----	-----	----	156	226	140*	25.0	64,200
8/28	0	-----	13,000	40,000	220	156	238	132*	26.5	62,100
9/7	0	95,000	5,600	21,000	10	164	248	92*	26.0	56,700
9/11	0	44,000	4,200	9,200	0	186	250	70*	26.5	56,000
9/18	0	240,000	2,400	23,000	122	128	186	1100*	22.5	87,900
9/25	9.1	64,000	5,300	44,000	0	140	198	600*	20.0	84,100
10/2	2	95,000	2,900	12,000	0	164	238	150*	19.0	64,400
10/10	7	-----	-----	-----	----	162	248	93*	15.0	61,800
10/17	7.3	69,000	1,500	6,400	0	164	244	04*	14.0	62,800
10/23	3	350,000	9,500	37,000	15	158	238	89*	14.0	63,400
10/30	2	55,000	40,000	6,100	2	158	234	97*	12.5	64,800
11/6	1	290,000	10,000	20,000	32	162	234	80*	13.0	65,900
11/13	0	18,000	9,600	26,000	0	156	236	68*	11.0	66,200
11/20	0	570,000	28,000	39,000	20	164	230	118*	5.0	67,500
11/27	5	380,000	2,000	2,200	72	164	240	95*	3.5	68,700
12/4	1	650,000	38,000	1,400	16	164	234	90*	1.5	65,500
12/11	2	59,000	11,000	3,000	0	180	256	60*	1.0	40,500
12/18	0	79,000	31,000	740	----	190	254	60*	2.0	37,300
12/27	2	530,000	9,800	27,000	16	190	250	50*	0.5	36,500

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-2. SUMMARY OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL DATA CORRESPONDING TO PRESETTLED MISSOURI RIVER WATER ENTEROVIRUS ASSAY DATA AT LEXINGTON, MISSOURI

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. °C	River Flow CFS
1976										
12/9	0	38,000	12,500	------	----	172	244	16	8.1	
12/15	0	18,000	2,900	20,000	----	194	270	11	8.0	
12/20	1	3,500	3,500	500	----	100	184	4.5	8.1	
1977										
1/5	0	1,300	0	------	----	198	268	8.6	8.1	
1/11	8	------	1,200	4,000	----	214	284	3.1	7.9	
1/18	6	1,600	850	------	15	218	290	2.6	7.6	
8/17	0	31,500	4,100	25,000	11					
8/22	1	154,000	3,800	------	16	120	204	14	8.0	
9/9	0	40,500	5,300	860,000	86	118	144	125	8.0	
10/20	1	495,000	8,400	74,000	40	168	240	55	8.2	
10/21	0	------	------	------	----					
10/25	0	245,000	19,000	257,000	610	122	166	180	8.1	
10/26a	1	312,000	11,000	37,000	415	134	184	155	8.2	
10/26b	0	600,000	13,000	118,000	489	140	182	145	7.8	
10/27	0									
1978										
3/7	0	------	------	------	----					
3/9	6	------	------	------	----					
3/10	0	------	------	------	----					
3/13	5	------	------	------	----					
3/14	0	------	------	------	----					
3/22	10	------	------	------	----					

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-3. SUMMARY OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL DATA CORRESPONDING TO LIME SOFTENED MISSOURI RIVER ENTEROVIRUS ASSAY DATA AT LEXINGTON, MISSOURI

Date	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	River Temp. ^oC	River Flow CFS
1976										
12/2	0	0	0	60	----	70	140	16	10.0	
12/10	0	0	0	-----	----	164	70	27	10.1	
12/16	0	0	0	-----	----	98	176	5.0	9.8	
12/21	0	12,600	0	-----	----	100	184	4.5	9.9	
1977										
1/6	0	4,500	0	110	----	100	178	4.2	9.6	
1/12	5	800	70	950	----	102	176	5.5	10.1	
1/20	2	4,200	200	100	5	100	188	1.7	9.3	
4/6	0	0	0	390	0	78	128	22	9.4	
8/9a	0									
8/9b	0	10	0	1,700	0	54	110	15	10.1	
8/17a	0									
8/17b	0	0	0	370	2					
8/23a	0									
8/23b	0	0	0	627	0	50	171	8.0	10.1	

APPENDIX C-3 (continued)

Date	Sample Type	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	pH
1/17	Raw Mo. River	38	17,000	1,900	-----	15	218	290	5.0	8.0
1/18	Presettled	6	1,600	850	-----	15	218	290	2.6	7.9
1/20	Lime Softened	2	4,200	200	100	5	100	188	1.7	9.3
1/21	Finished	0	0	0	5	0	90	184	0.11	9.1
8/16	Raw Mo. River	1.6	134,000	10,000	185,000	59	128	210	205	
8/17	Presettled	0	31,500	4,100	25,000	11				
8/17	Lime Softened	0	0	0	370	2				
8/18	Finished	0	-----	-----	-----	----				
8/22	Raw Mo. River	0	108,000	17,500	-----	16	124	208	205	8.0
8/23	Presettled	1	154,000	3,800	-----	16	120	204	14	8.0
8/24	Lime Softened	0	0	0	627	0	50	171	8.0	10.1
8/25	Finished	0	0	0	7	0	41	149	0.08	9.3

* indicates turbidity data obtained from Lexington Water Treatment Plant in JTU's

APPENDIX C-4. SUMMARY OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL DATA CORRESPONDING TO ENTEROVIRUS REMOVAL BY CONVENTIONAL WATER TREATMENT OPERATIONS AT LEXINGTON, MISSOURI

Date	Sample Type	Virus Ct. PFU per 100 gal	Total Coliforms No./100 mℓ	Fecal Coliforms No./100 mℓ	Std. Plate Count No./mℓ	Yeast Count No./100 mℓ	Alkalinity mg/ℓ as $CaCO_3$	Hardness mg/ℓ as $CaCO_3$	Turbidity NTU*	pH
1976										
12/6	Raw Mo. River	0	28,400	6,000	-----	----	174	248	70	8.0
12/9	Presettled	0	38,000	12,500	-----	----	172	244	16	8.1
12/10	Lime Softened	0	0	0	-----	----	164	70	27	10.1
12/13	Finished	0	0	0	-----	----	86	---	0.06	9.7
12/14	Raw Mo. River	6	21,300	9,300	20,000	----	188	266	43	8.0
12/15	Presettled	0	18,000	2,900	-----	----	194	270	11	8.0
12/16	Lime Softened	0	0	0	-----	----	164	176	5.0	9.8
12/17	Finished	0	0	0	500	----	90	180	0.08	9.6
12/18	Raw Mo. River	10	314,000	20,200	500	----	180	260	50	7.9
12/20	Presettled	1	3,500	3,500	500	----	194	270	11	8.0
12/21	Lime Softened	0	12,600	0	-----	----	100	184	4.5	9.9
12/22	Finished	0	-----	-----	-----	----				
1977										
1/4	Raw Mo. River	4	84,000	0	-----	----	198	268	33	8.0
1/5	Presettled	0	1,300	0	-----	----	198	268	8.6	8.1
1/6	Lime Softened	0	4,500	0	110	----	100	178	4.2	9.6
1/8	Finished	0	0	0	25	----	98	192	0.40	9.5
1/10	Raw Mo. River	---	-----	-----	-----	----	200	280	25	8.0
1/11	Presettled	8	-----	1,200	4,000	----	214	284	3.1	7.9
1/12	Lime Softened	5	800	70	950	----	102	176	5.5	10.1
1/13	Finished	0	0	30	-----	----	92	178	0.14	9.6

APPENDIX D. MS_2 BACTERIOPHAGE DIE-AWAY STUDIES CONDUCTED WITH THE MOBILE PILOT PLANT AT LEXINGTON, MISSOURI

1. Recovery of MS_2 bacteriophage following addition of 50 ml of MS_2 concentrate (assayed at $5x10^{11}$ phage/ml) to 175 gallons of presettled Missouri River water.

Time after MS_2 addition, minutes	MS_2 Bacteriophage Recovered (phage/ml)		
	Experiment 1		Experiment 2
0.25	2		1
0.50	0		4
1	1		2
2	2		0
5	4		5
10	0		1
20	3		2
30	2		4
45	0		2
60	2		0
120	4		1
Ave. 1.8	1.8	Ave. 2.0	2.0

Results indicate that die-away occurs almost instantaneously. Surviving phage particles appear to be stable over several hours.

Based on the average MS_2 bacteriophage concentration recovered, an estimate of the percent survival can be calculated:

$$1.9 \text{ phage/ml } (175 \text{ gal})(3.78 \text{ l/gal})(1000 \text{ ml/l}) = 1.27x10^6 \ MS_2 \text{ phage surviving}$$

$$\% \text{ survival} = \frac{1.27x10^6}{(50)(5x10^{11})} = 5.1x10^{-6} \%.$$

APPENDIX D(continued)

2. Recovery of MS_2 bacteriophage following addition of 50 ml of MS_2 concentrate (assayed at $7x10^{11}$ phage/ml) to 175 gal of presettled Missouri River water.

Time after MS_2 addition, minutes	MS_2 Bacteriophage Recovered (phage/ml)	
	Experiment 1	Experiment 2
0.25	7	0
0.50	0	11
1	10	8
2	3	8
5	0	5
10	7	0
20	0	15
30	5	7
45	8	1
60	3	0
120	8	10
Ave. 4.6 phage/ml	4.6 Ave. 5.5	5.5

$$\text{Percent lysis survival} = \frac{5.1\ \text{phage/ml}(175\ \text{gal})(3.78\ \text{l/gal})\ (1000\ \text{ml/l})}{(50)\ (7x10^{11})}$$

$$= 4.8x10^{-6}\ \%\ .$$